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ELECTROPHORETIC SEPARATION OF CELLS
AND PARTICLES FROM RAT PITUITARY

NASA GRANT - NAG8-953

MID TERM PROGRESS REPORT

BY

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I. INTRODUCTION

MID TERM PROGRESS REPORT

ANALYSIS OF PITUITARY CELL ELECTROPHORESIS EXPERIMENT DONE ON IML-2 (7/94)

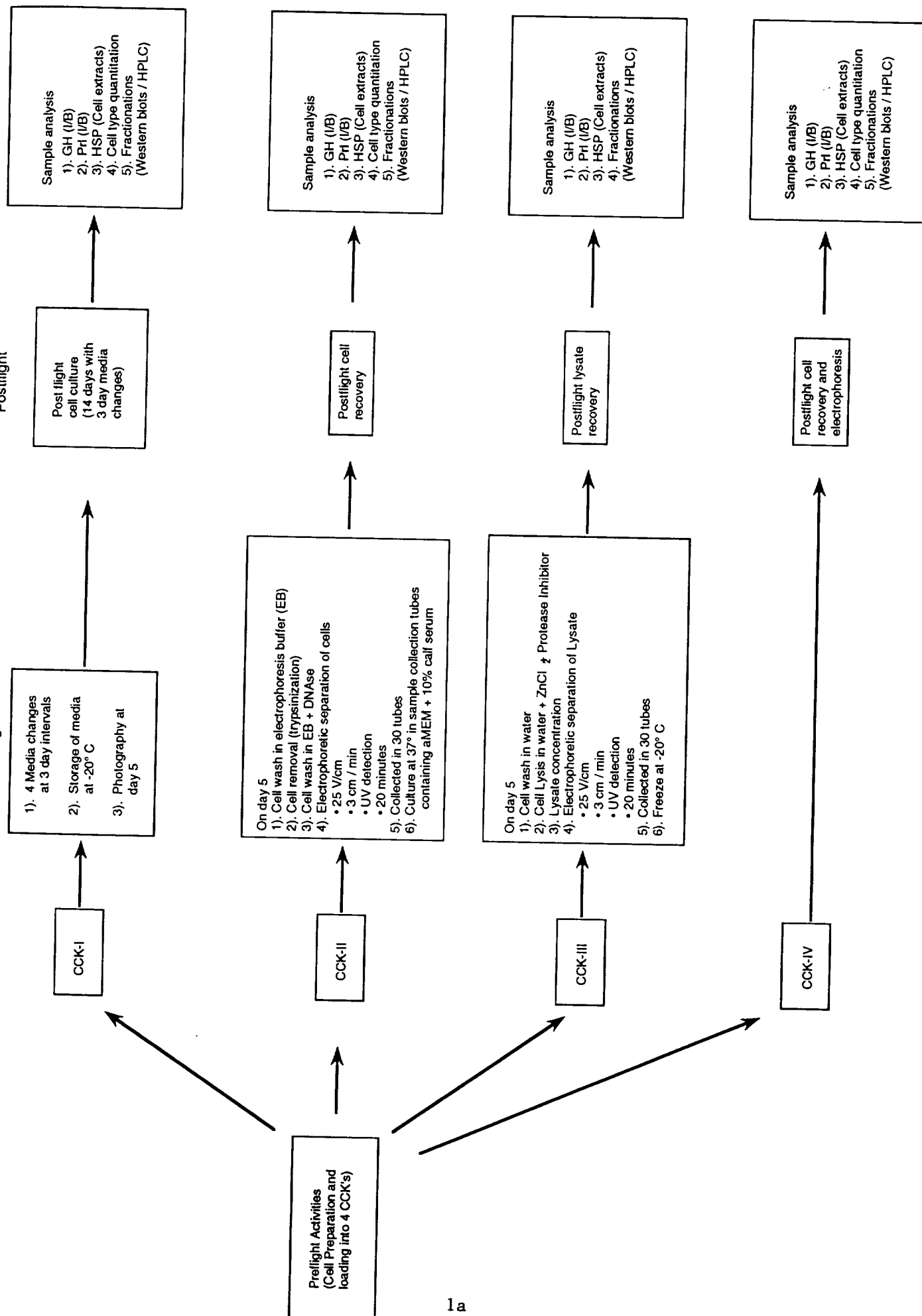
In spite of the fact that a vast majority of the electrophoresis effort (~90%) could not be done on this mission due to failure of FFEU hardware, we find some interesting differences in flight samples obtained from other parts of the experiment. These differences are entirely novel and sometimes unexpected. This report is organized into 4 parts. Each part describes the data collected thus far from each of the 4 cell culture kits (CCK) which flew in space. Each CCK was loaded with 40×10^6 fresh pituitary cells; all CCK's were identical at the start of the experiment because we prepared one pool of cells.

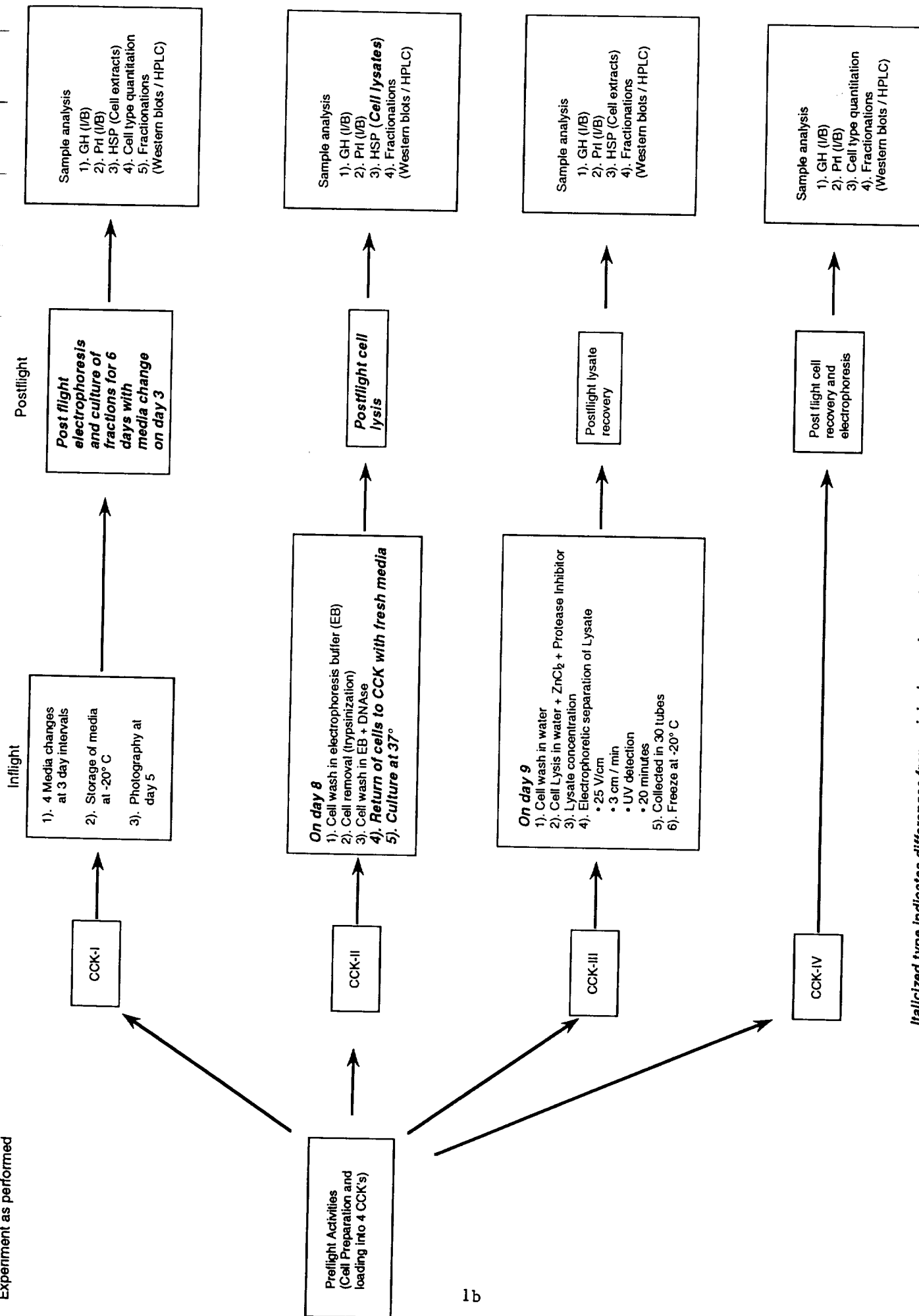
Design of Entire Experiment

The original design is shown on page 1a; modifications to this design were required due to the hardware failure. These modifications are highlighted on page 1b.

Inflight

Postflight





Italicized type indicates differences from original experimental plan.

II. CCK #1

CCK #1.

Cell culture manipulations in flight were carried out *exactly* as planned. Both the astronauts in space and our technical crew on the ground at KSC performed these tasks flawlessly. No contamination was encountered. Because cell electrophoresis was a focus of this experiment, a decision was made to remove the flight cells from CCK #1 and electrophorese them at KSC postflight. This was done to determine if microgravity had any effect on cell mobility.

Synchronous ground control cells from CCK #1 could not be electrophoresed because the ground unit at KSC failed after the flight cells were run (the ground unit clogged). Therefore, the asynchronous ground control portion of this entire experiment remains to be done.

The statements listed below are based on the data obtained from samples prepared from CCK #1. A battery of analytic techniques is being used. These include (1) growth hormone (GH) immunoassay, (2) GH bioassay (bone growth in live animals); (3) HPLC (size exclusion and ion exchange); (4) image analysis of cells (flow cytometry/immunocytochemistry/cytoplasmic area hormone occupancy).

Key results thus far show that:

- In flight, cells released more immunoreactive growth hormone (GH)/day than controls (pg. 6 top).
- In flight, cells sometimes released more biologically active GH than controls (pg. 6 bottom) except in the day 2 and 11 sample. Recall that the day 2 sample actually represents 5 days of culture (i.e. 3 days pre-flight and 2 days in flight). The major loss of bioactive GH (6x) in the flight sample at day 2 (But no loss in immunoactivity) could be due to suppression induced by stress of entry into microgravity(?). Clearly, the cells are capable of secreting quality hormone in flight (i.e. they recover from this presumed entry stress). Note however that reentry had no effect on release of bioactive GH.
- Analysis of culture media by HPLC gel exclusion indicates that the major peak of immunoreactive GH (iGH) is between 59,000 and 18,000 MW (i.e. fractions 15-20, the size of monomeric and dimeric GH). Higher MW GH (>59K, fractions 7-14) is probably due to association of monomer/dimer GH with serum proteins in the culture medium. In virtually all cases the amounts of iGH were greater in F than G (pg. 7). Samples were too dilute to permit testing for biological activity.
- Analysis of culture media by HPLC ion exchange chromatography showed that all of the iGH emerged from the column quickly, indicating apparent highly positively charged species. Flight had no effect on this general behavior (pg. 8); however, note that the amounts of iGH recovered from flight samples, relative to ground, were often quite different.

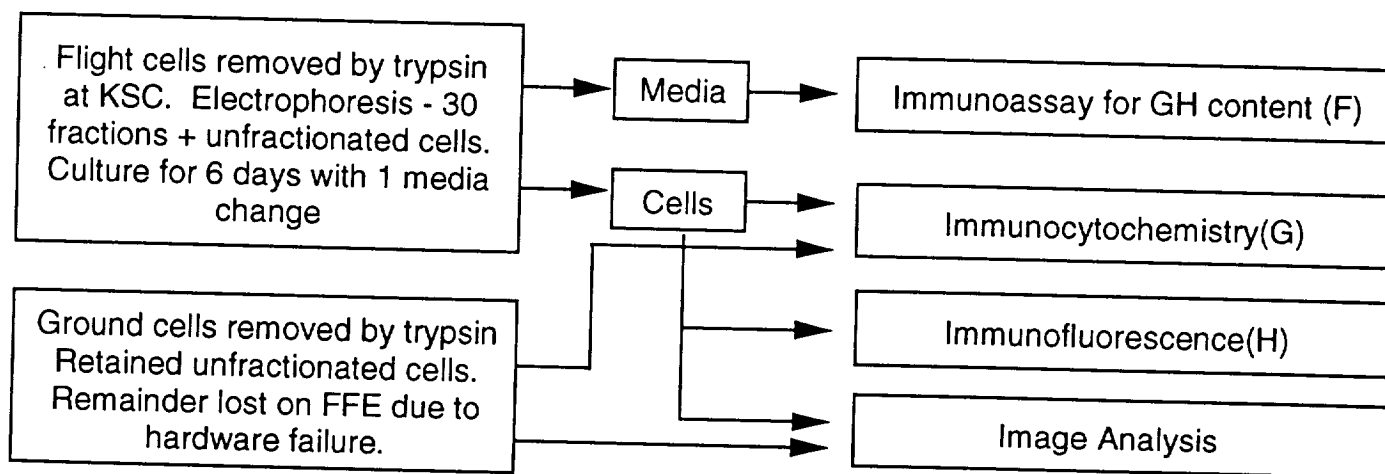
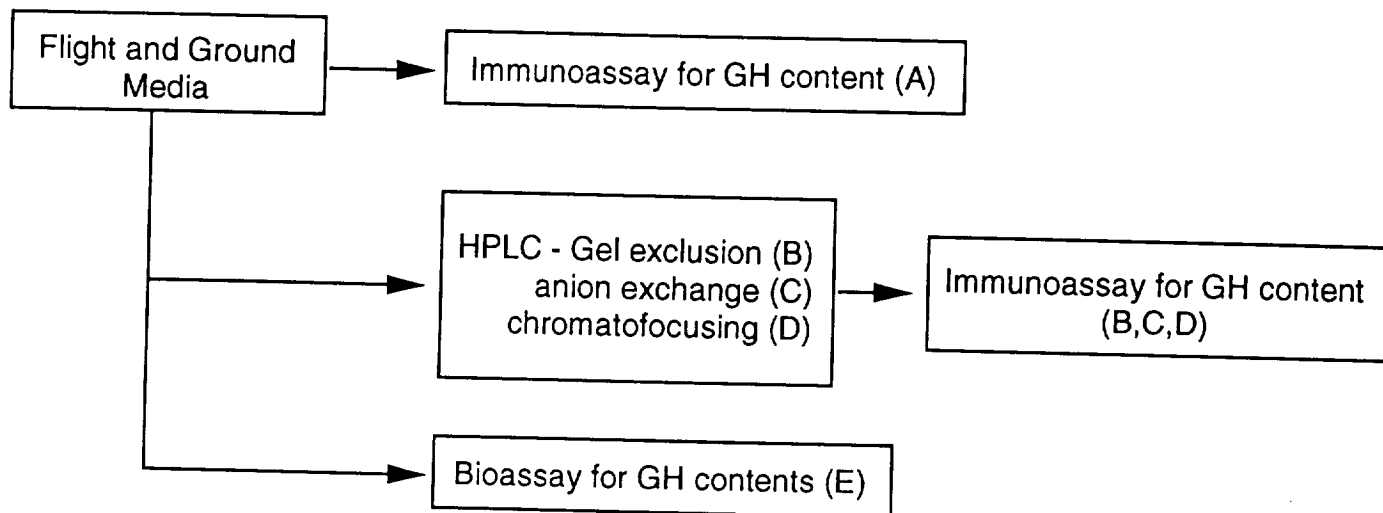
- Analysis of the culture media by HPLC chromatofocusing indicates that flight had no major effect on the isoelectric point(s) of released iGH (pg. 9).
- Postflight recovery of flight cells from CCK #1 by trypsinization at KSC yielded healthy cells which were electrophoresed on a small FFEU made for us by NASDA. The distribution profiles of total cell population (- □ -) and GH cells - ■ -) indicate that (1) anodal migration had occurred; (2) that some evidence for GH cell enrichment was found, but this was only modest at best (pg. 14). The most interesting finding was that mobility of flight cells from CCK #4 (see section 4) was very different from those in CCK #1. This result suggests either the frequency of medium change affects cell migration or (2) that flight (in conjunction with medium change frequency) affects cell migration. This issue will not be resolved further until we do the asynchronous ground control experiment (pg. 10).
- Some of the cells from flight CCK # 1 were also cultured at KSC after electrophoresis. Culture was for 6 days with no media change. Analysis of the 6 day culture media clearly show that viable hormone secreting cells were present after flight and postflight electrophoresis (pg. 10 bottom). Note that hormone producing cells are enriched in certain regions.
- Flow cytometric profiles of ground and flight cells from CCK #1 are complex (pgs. 11/12) and their full interpretation will not be given here. However, a major difference between the microscopic appearance of cells in CCK #1 vs. #4 was the marked proliferation of pituitary fibroblasts in CCK #1 but not in CCK #4. Our 3 month report to Bob Snyder offered photos of this difference. It is confirmed by the DNA distribution profiles shown on pgs. 11/12 vs. equivalent profiles from CCK #4 on pgs. 6/7 (box marked X).

The percentage area of the cytoplasm in a GH cell occupied by GH was studied on cells from CCK #1 using techniques we have developed in previous spaceflight experiments. The data on pg. 13 were collected on 200 cells/group on coded slides to avoid investigator bias. Note that ~40% of the cytoplasm of the GH cell is occupied by hormone at the beginning of the experiment (see "starts" - pg. 13). After the 14 day culture period, GH cells from both ground CCK #1 and CCK #4 had significantly lower hormone area occupancy than that initially present. However, this reduction did not happen when cells were cultured for 14 days in conventional T25 flasks. This result shows that biophysical/cytoplasmic organizational character of the GH cell on the polycarbonate surface of the CCK is very different from that on conventional plastic surfaces. Flight caused an additional (and statistically significant, $p < 0.02$) reduction in cytoplasmic area hormone occupancy that was made even more obvious by each of media changeouts.

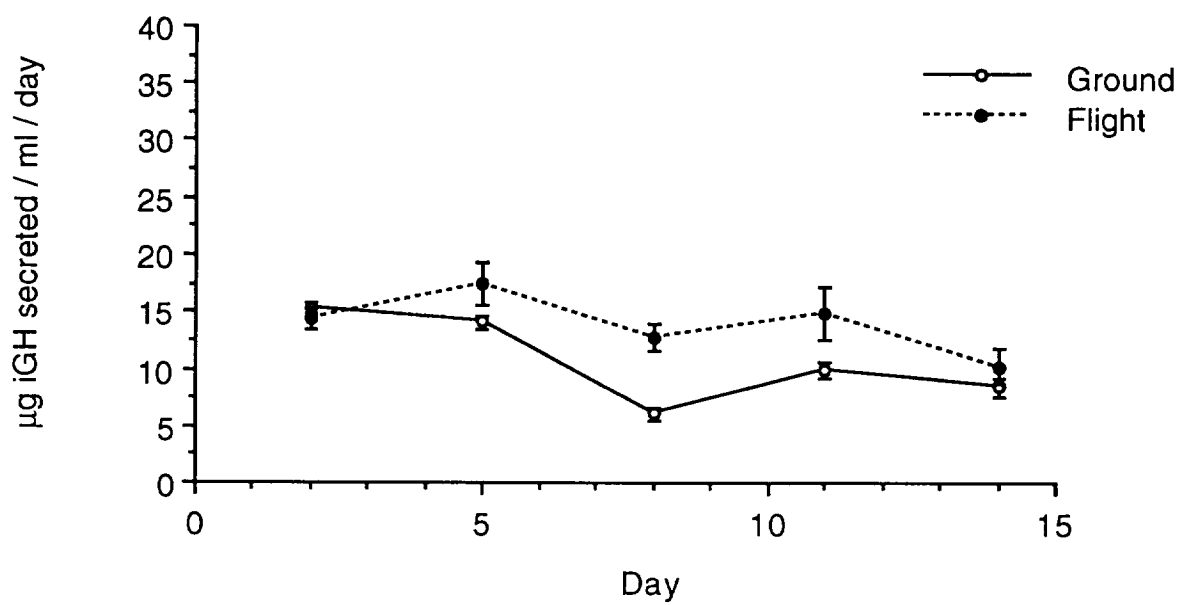
Activities in Microgravity

| Proposed | Actual | % of objective achieved |
|--|---|-------------------------|
| CCK #1 | | |
| • Change out of media 5 X | Media changed out 5 X | 100% |
| • Freeze spent media | Media frozen | 100% |
| • Maintain ground operations identical to flight (eg. media change outs, temperature) | Operations at KSC were done within 15 minutes of space ops. Incubator temp within 0.2° C of flight unit. | 100% |
| • Remove culture media on landing | Media removed | 100% |
| • Continue culture for 14 days on ground with media changes. then extract intracellular hormone. | Flight cells recovered. Some of the cells were cultured and the rest subjected to FFE at KSC followed by 6 days in culture with media change at day 3. Cells were then stained for intracellular GH. Ground control cells were processed as the flight but failure of the FFE and loss of the cells precluded separation and continued culture. Unseparated cells were stained for intracellular GH. | 5% |

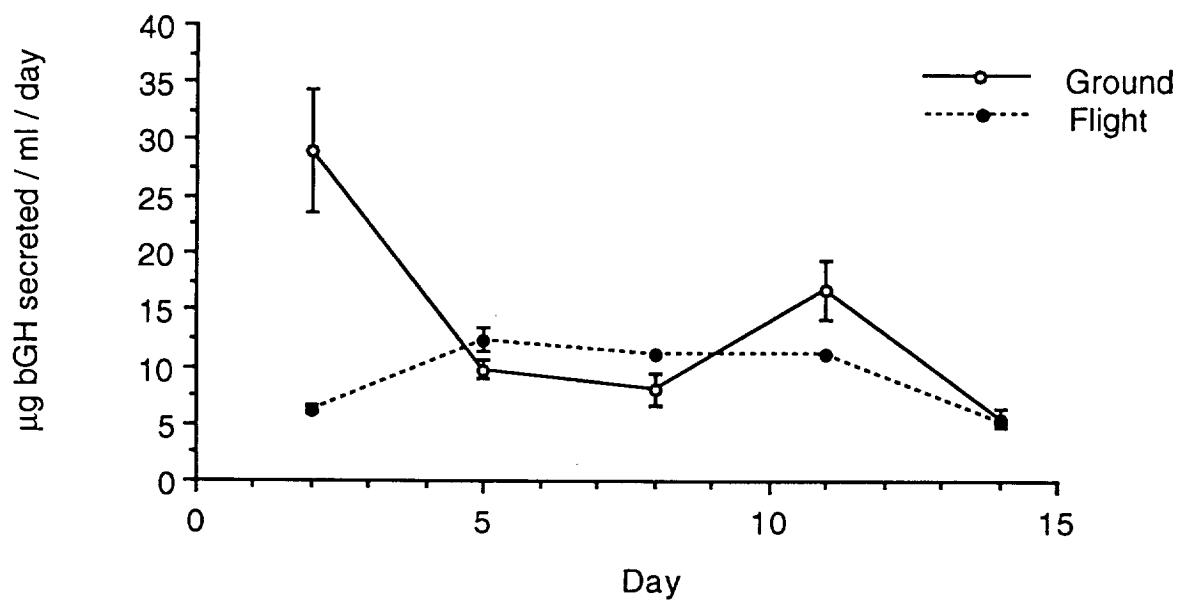
CCK #1 Growth Hormone Analysis



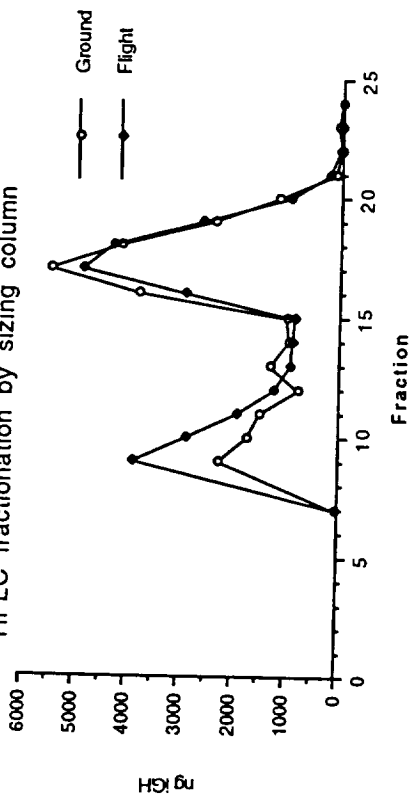
CCK #1
Media exchange
Immunoactive Growth Hormone secreted / ml / day



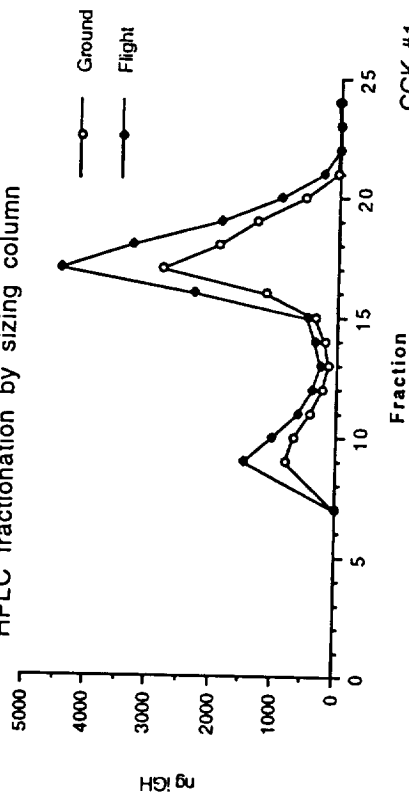
CCK #1
Media exchange
Bioactive Growth Hormone secreted / ml / day



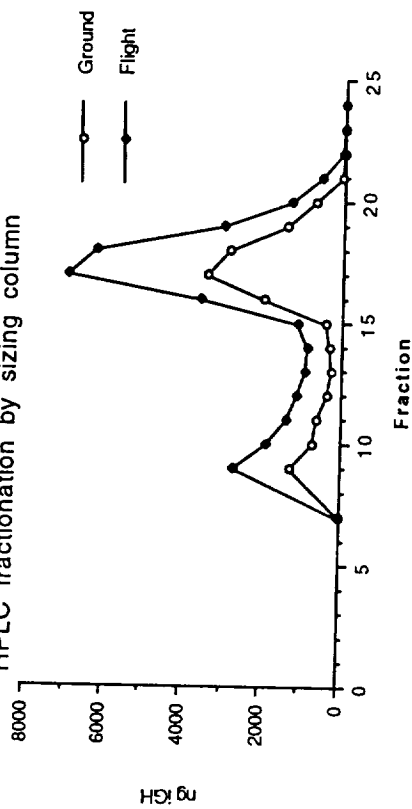
CCK #1
Media changeout on mission day 2
HPLC fractionation by sizing column



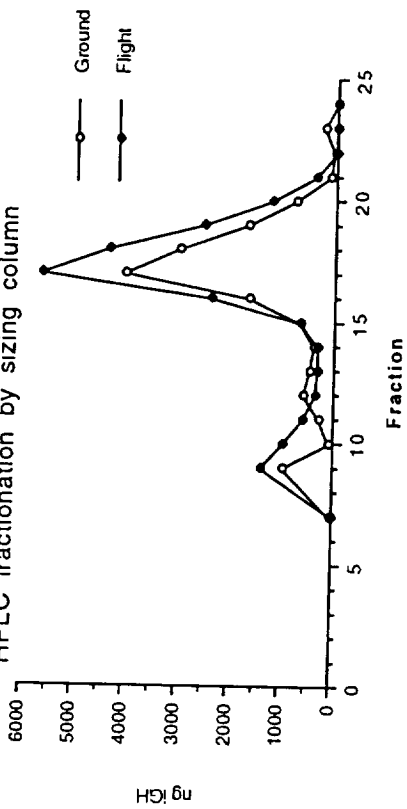
CCK #1
Media changeout on mission day 8
HPLC fractionation by sizing column



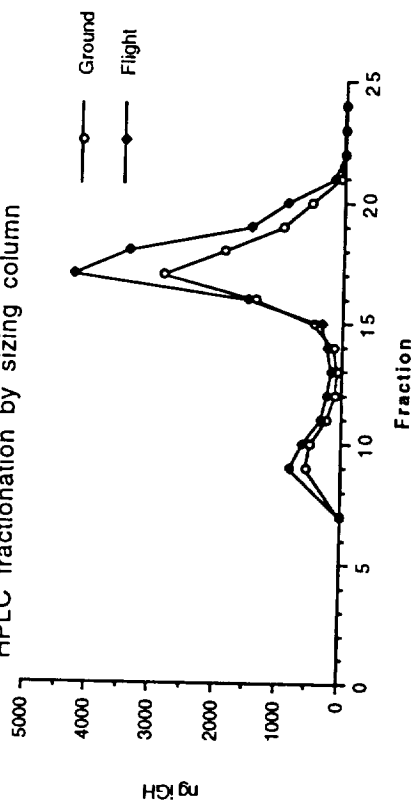
CCK #1
Media changeout on mission day 5
HPLC fractionation by sizing column



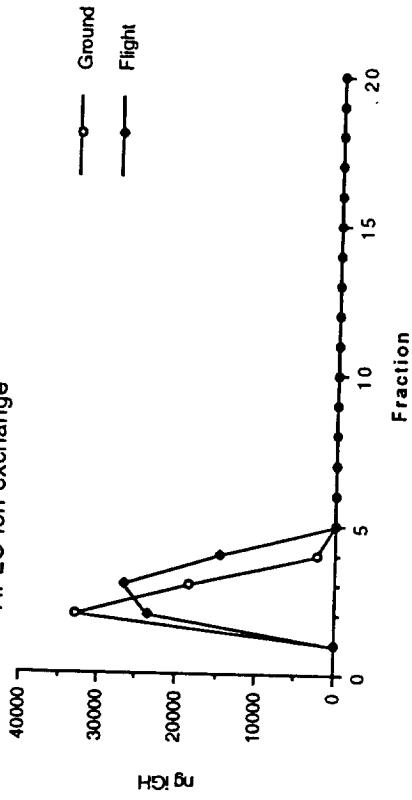
CCK #1
Media changeout on mission day 11
HPLC fractionation by sizing column



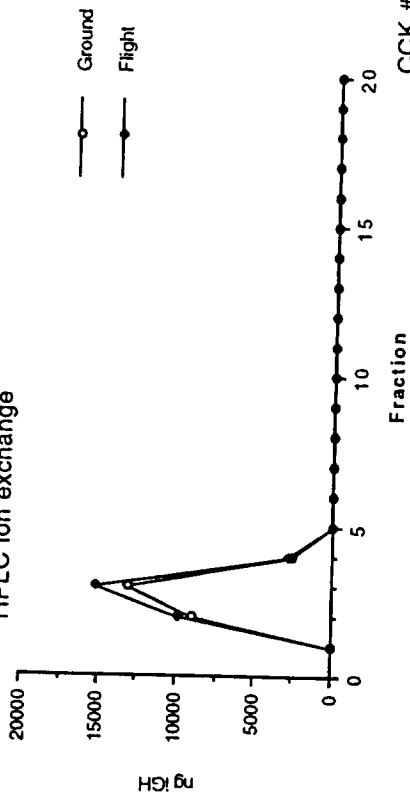
CCK #1
Media changeout on mission day 14 (landing)
HPLC fractionation by sizing column



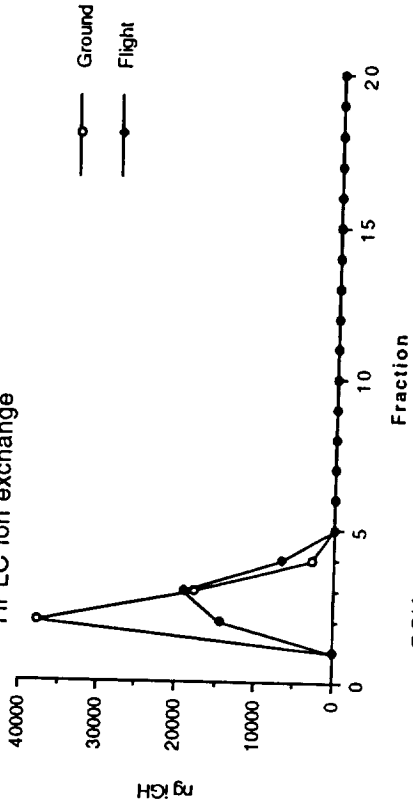
CCK #1
Media changeout on mission day 2
HPLC Ion exchange



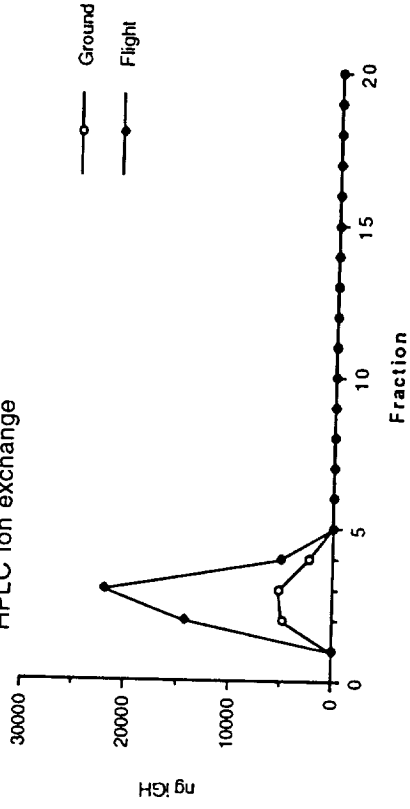
CCK #1
Media changeout on mission day 8
HPLC Ion exchange



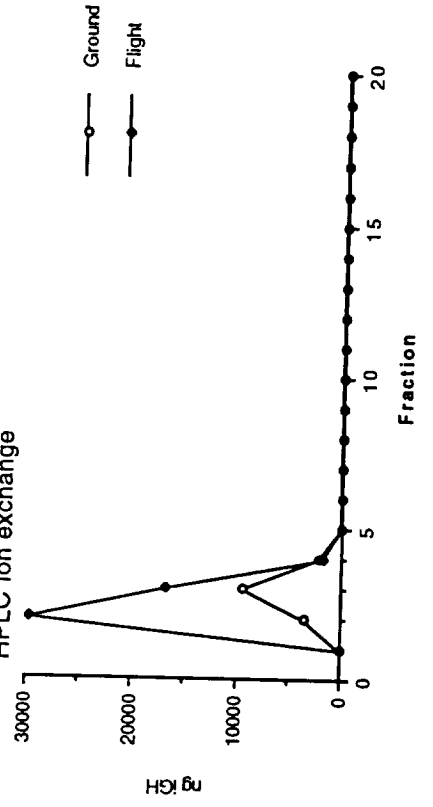
CCK #1
Media changeout on mission day 5
HPLC Ion exchange

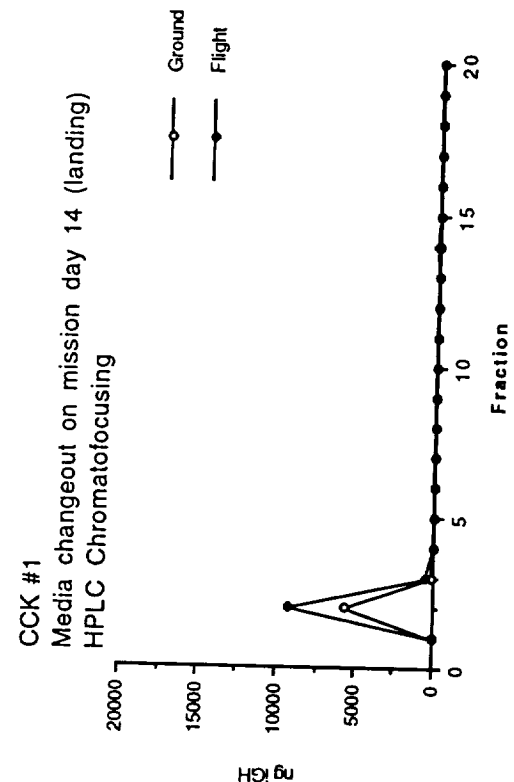
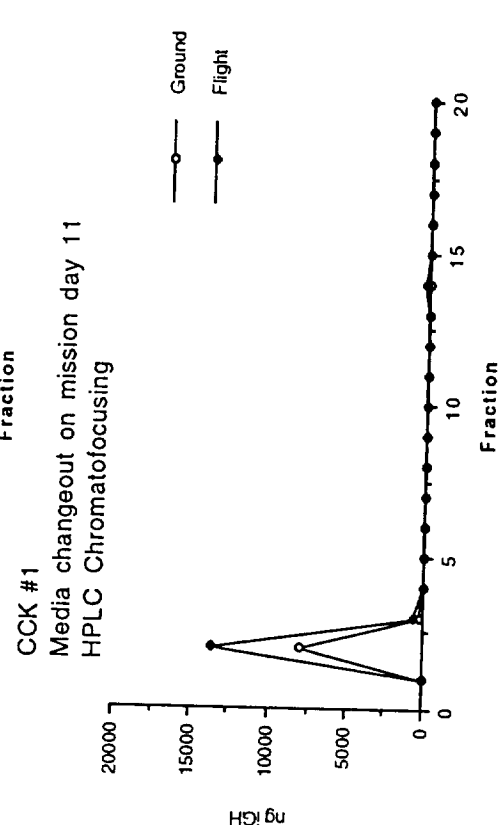
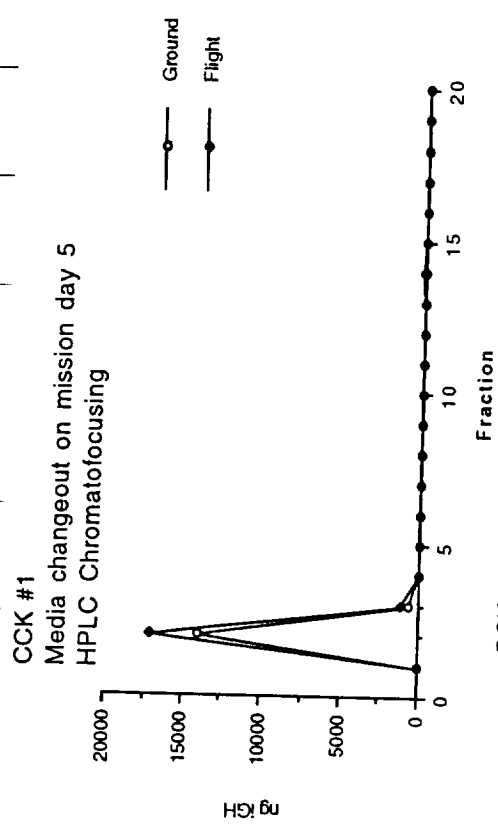
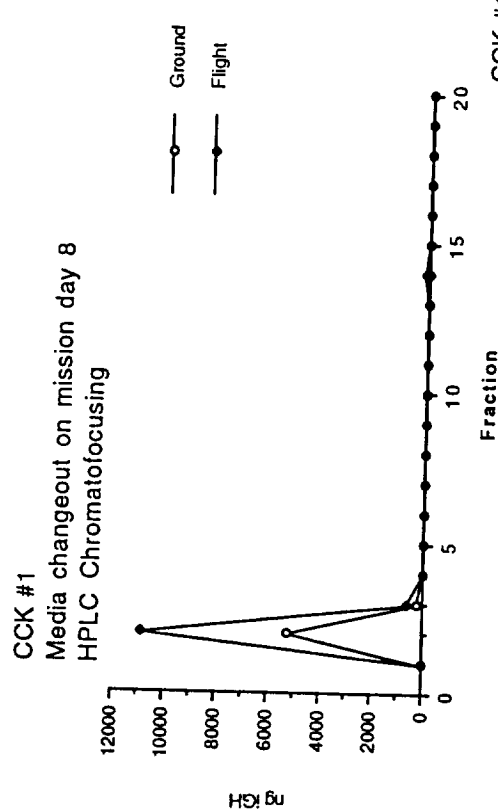
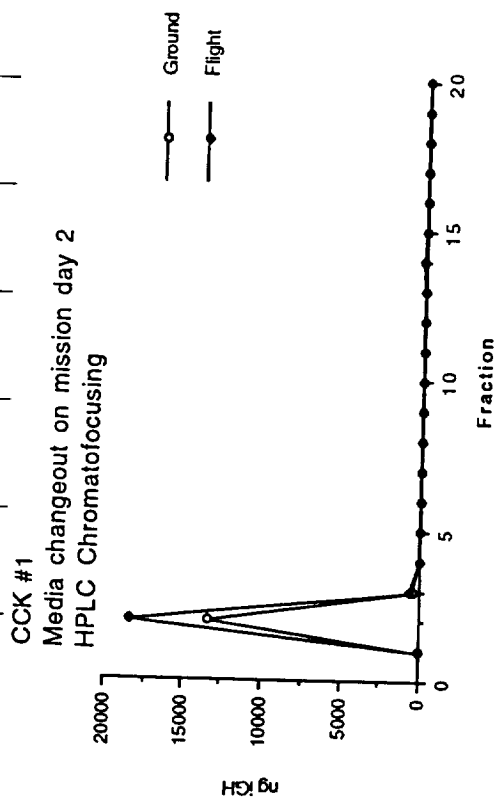


CCK #1
Media changeout on mission day 11
HPLC Ion exchange

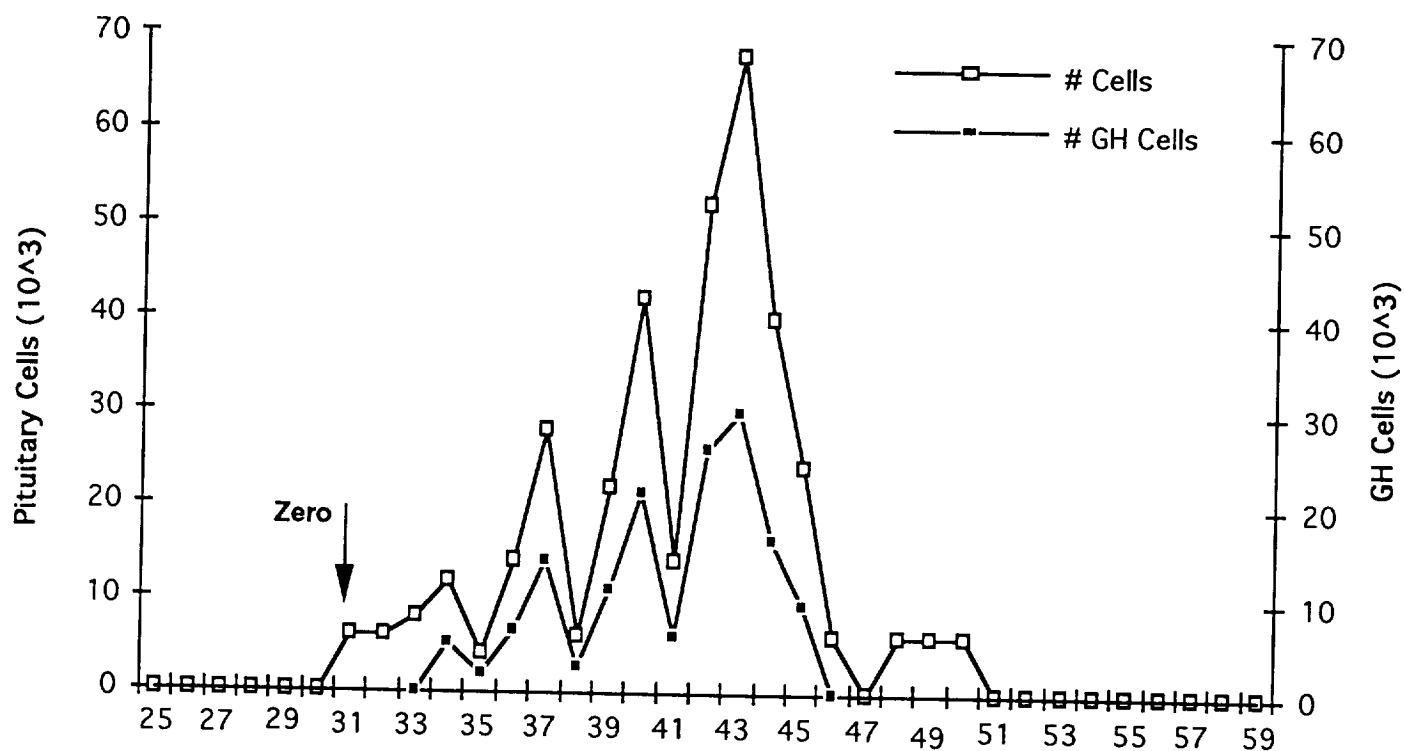


CCK #1
Media changeout on mission day 14 (landing)
HPLC Ion exchange

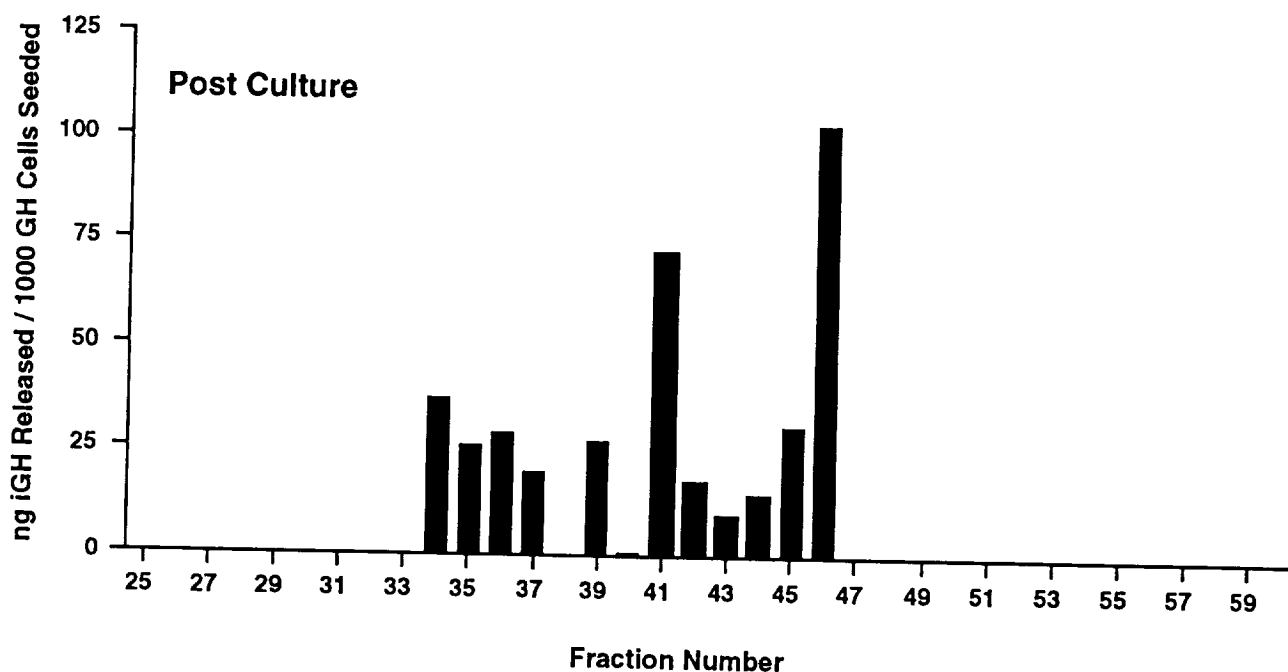




Flight CCK 1 Pituitary Cell Run Profile from ground Free Flow Electrophoresis Unit



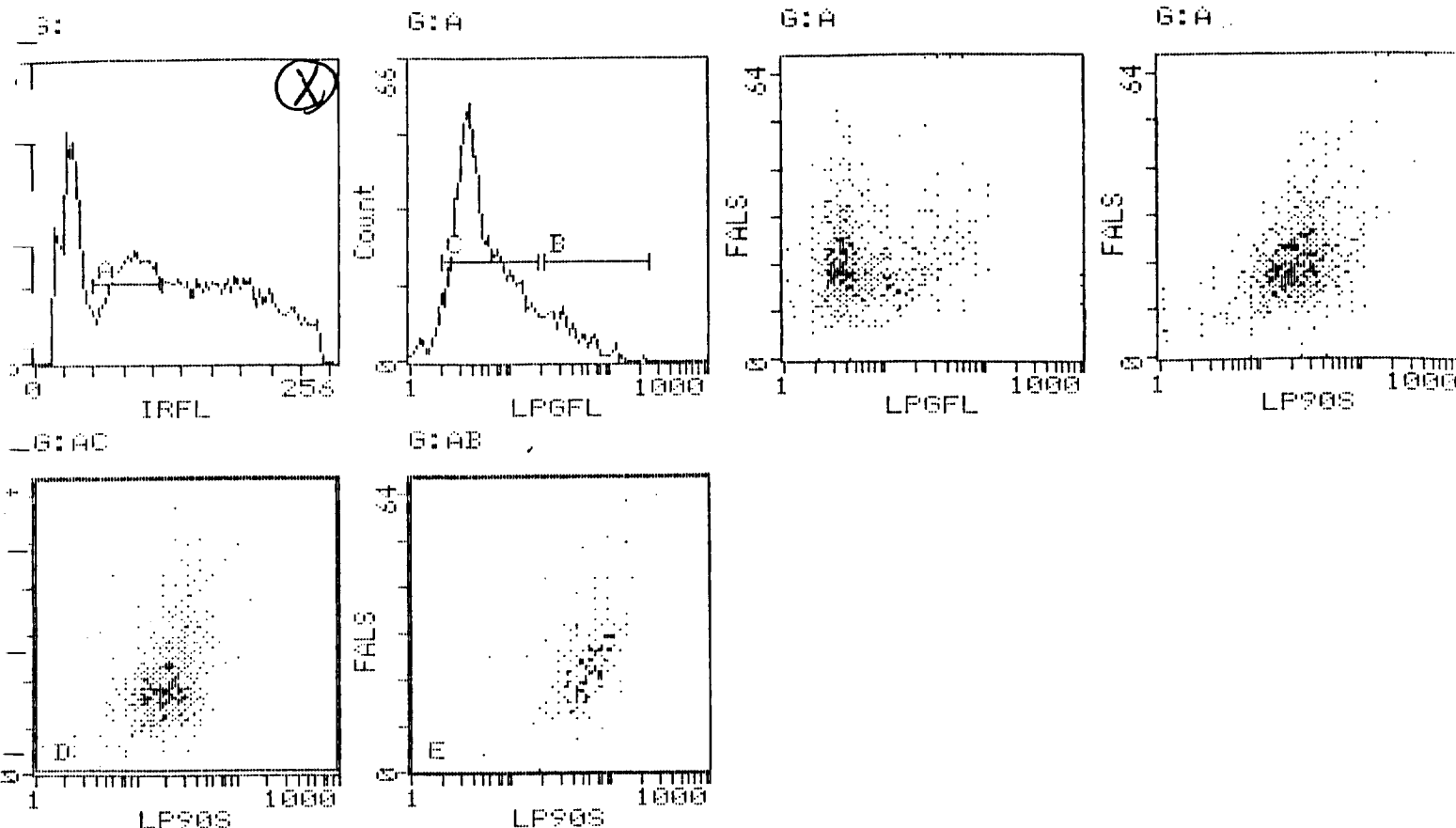
Release of iGH from Flight Cells of CCK 1 after Ground Free Flow Electrophoresis and Ground Cell Culture



FILE NAME: IML2

GROUND

SAMPLE DATE: 25Oct94



----- SAMPLE INFO -----

| | | | | |
|---------------|-----------|-------------|-------------|----------------|
| PROTOCOL | : TOM | | LAST HIST | : |
| DATA RATE | : Unknown | TOTAL COUNT | : 10,001 | |
| INSTRUMENT | : MDADS | | LIST FILE | : 251094P4.L04 |
| SAMPLE NAME | : IML2 | | PROT FILE | : T0000007.PRO |
| SAMPLE NUMBER | : 4 | | SAMPLE DATE | : 25Oct94 |
| COMMENTS | : G1M | | SAMPLE TIME | : 00:24 |

----- STATISTICS -----

DUAL PARAMETER STATISTICS

| ID | Cent | Area | Position | Height | Mean | SD | CV | Mean | SD | CV |
|----|------|------|----------|--------|------|------|------|------|------|------|
| | 100 | 2179 | 19, 18 | 18 | 19.5 | 13.4 | 68.6 | 20.6 | 8.9 | 43.3 |
| | 100 | 402 | 50, 19 | 6 | 58.5 | 35.7 | 60.9 | 24.1 | 10.4 | 43.0 |

D Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D

E Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

FILE NAME : IML2
 SAMPLE NUMBER: 4

ANALYSIS

FLIGHT

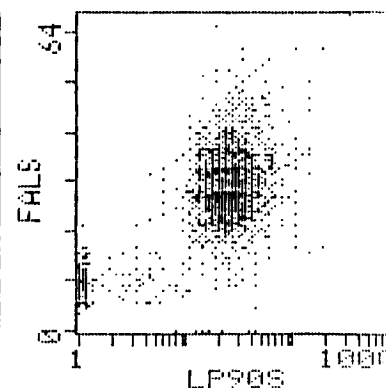
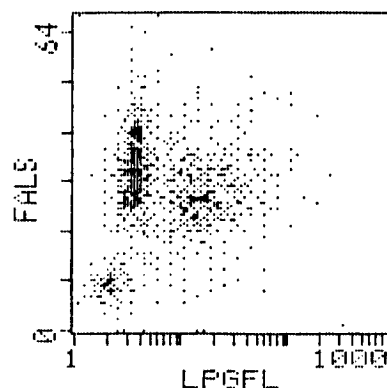
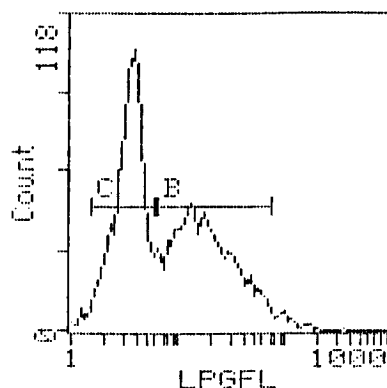
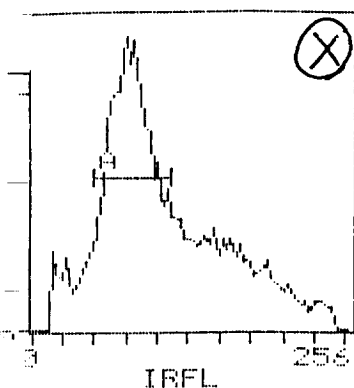
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G:

G:A

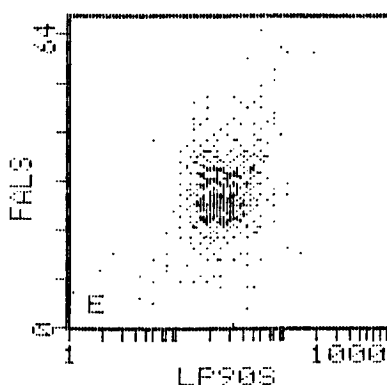
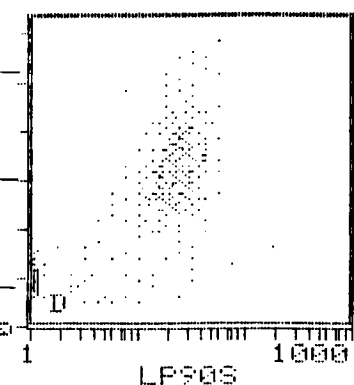
G:A

G:A



G:AC

G:AB



SAMPLE INFO

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 DATA RATE : Unknown
 INSTRUMENT : MDADS
 SAMPLE NAME : IML2
 SAMPLE NUMBER: 6
 COMMENTS : F1P

TOTAL COUNT : 10,001

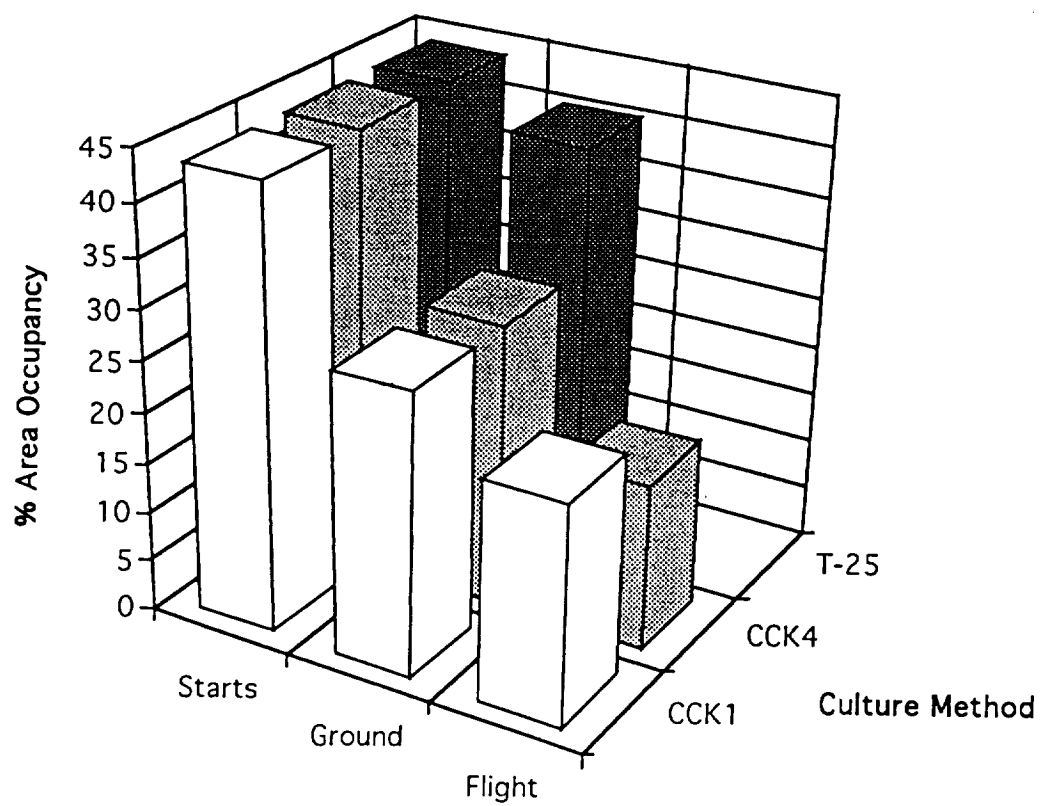
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 PROT FILE : T00000007.PRO
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 SAMPLE TIME: 00:30

STATISTICS

DUAL PARAMETER STATISTICS

| ID | Pcnt | Area |Peak..... | |X Channel.... | | |Y Channel.... | | |
|--|------|------|----------------|--------|-------------------|------|-------|-------------------|------|------|
| | | | Position | Height | Mean | SD | CV | Mean | SD | CV |
| D | 100 | 2561 | 1.1, 11 | 29 | 13.2 | 16.5 | 125.1 | 27.6 | 12.6 | 45.7 |
| E | 100 | 2715 | 23, 23 | 20 | 27.1 | 17.0 | 62.8 | 27.5 | 8.5 | 31.1 |
| Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D | | | | | | | | | | |
| Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E | | | | | | | | | | |

IML-2 Pituitary Cell Area Occupancy



Summary of CCK #1. Results to Date: The Bottom Line and Tentative Conclusions.

- The "stress" of getting into orbit affects the output of bioactive GH.
- Spaceflight appears to modify charges, but not the size, of the secreted immunoreactive GH.
- Cell growth behavior on the polycarbonate surface of the CCK is different than on conventional plasticware.
- Media changes in CCK #1 (on both ground and flight units) resulted in vigorous fibroblast growth that was not seen when media changes were not done.
- The frequency of medium changes affects electrophoretic mobility; we do not yet know if this is a flight effect.
- Frequency of cell feeding on the amount and activity of growth hormone (GH) released from cells in the different CCK's.

The original design of this experiment allowed us to determine microgravity's effect on hormone production as a function of the number of cell culture media changes done during the 14 day flight. It is important to indicate that all of the bioassay data in this report were provided by Dr. R. Grindeland of NASA Ames. These assays are involved in that they require large numbers of animals which have had their pituitary glands surgically removed. Thus far, 180 assay animals have been used. Based on data collected from CCK #1 (4 media changes); CCK #2 (1 medium change) and CCK #4 (0 change), the results thus far allow us to make the following conclusions (see pg. 16):

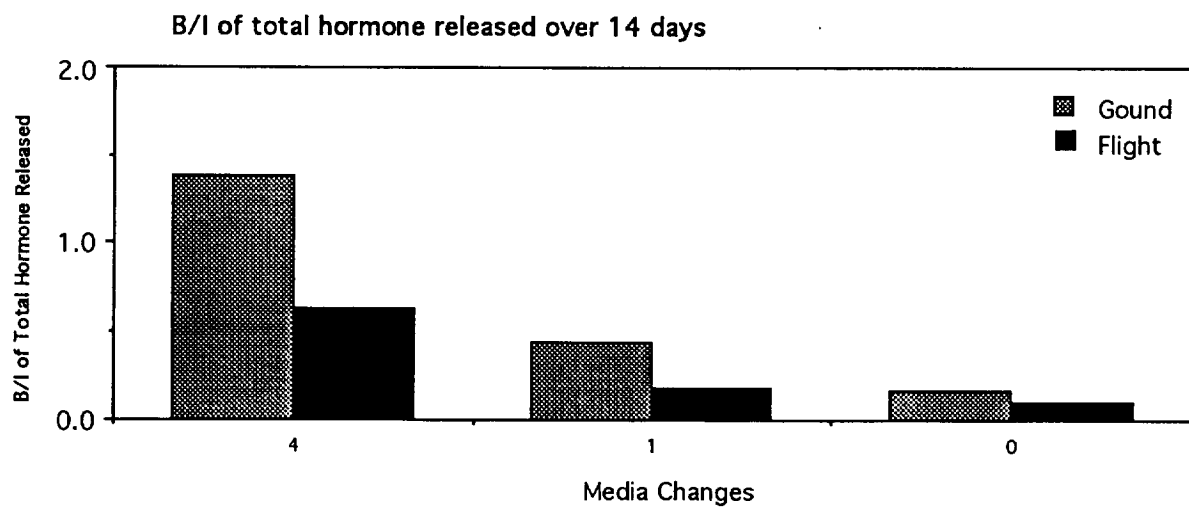
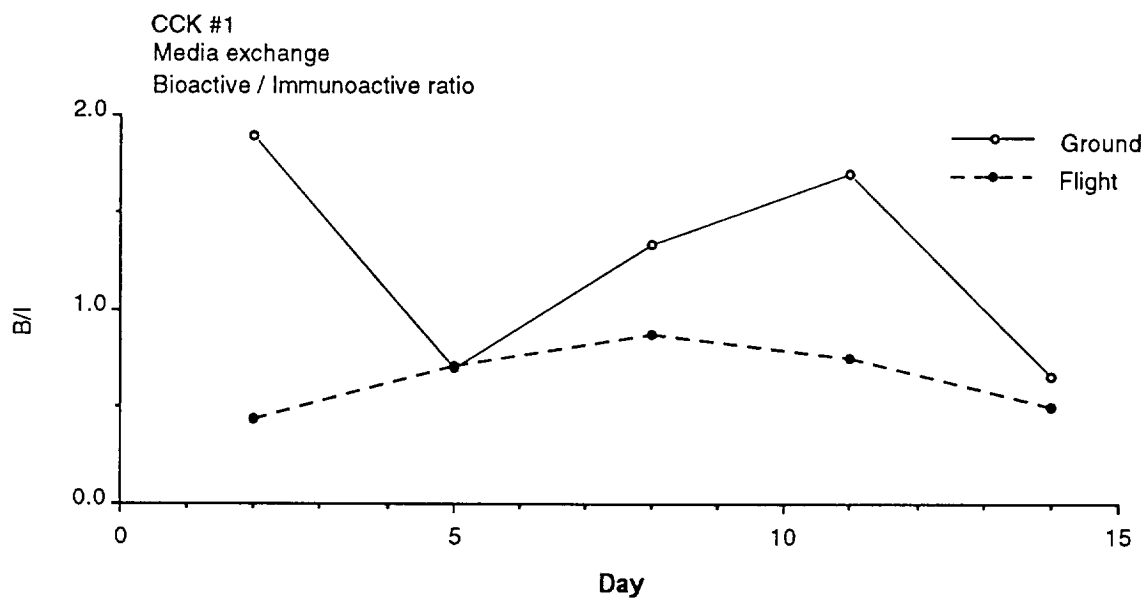
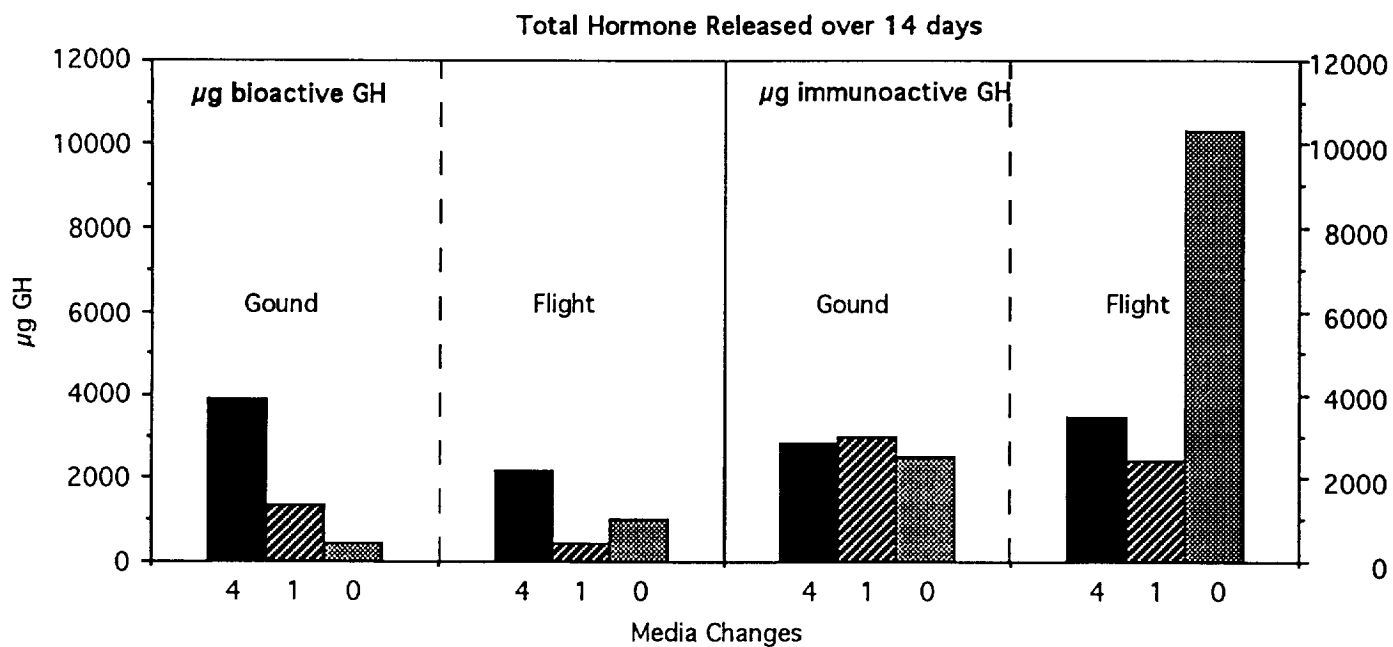
- On Earth, the amount of bioactive GH (bGH) released is directly related to the number of media changes performed.
- In microgravity, the amount of bGH released is reduced by ~50% (relative to ground controls) when media changes are done.
- Media change frequency in microgravity has relatively little effect on the immunoreactive GH (iGH) but
- When media changes are not done (CCK #4), flight GH cells behave very differently. Spaceflight accentuates the production of iGH. Sometime during the 14 day culture period they apparently "convert" to producing GH that is more immunoreactive. This is not true for ground cells.

- Spaceflight negatively affects the quality of hormone released. Thus the biological/immunological (B/I) activity ratio of the total secreted GH is usually reduced by at least 50% (bottom panel, pg. 16).
- Variables of both media change frequency and spaceflight affect the B/I of GH released.
- From CCK #1 data it appears that the cyclical nature of GH quality (i.e. B/I) secreted on the ground disappears in spaceflown cells (middle panel, pg. 16).

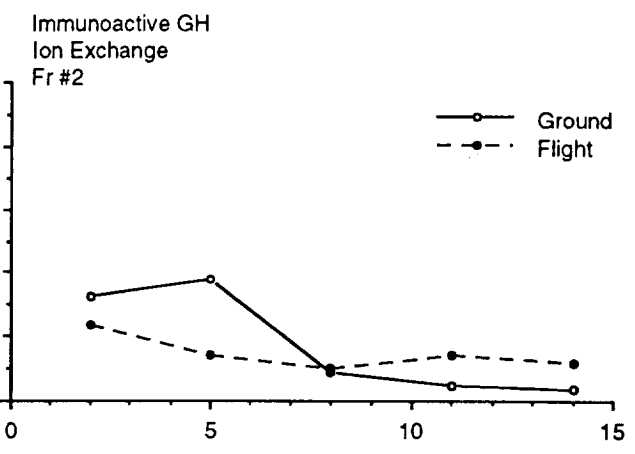
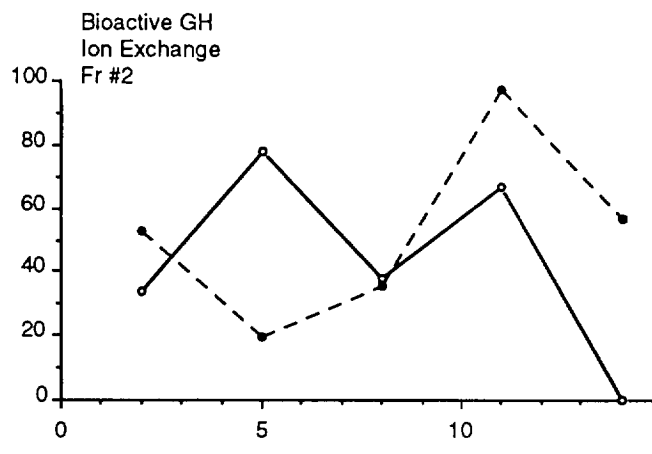
Characterization of GH released from CCK #1 by HPLC Ion Exchange Chromatography

We were somewhat surprised by the elution profiles of iGH which are summarized on page 8. These profiles suggested that the iGH was highly positively charged in both flight and ground samples, but that spaceflight might affect the concentrations of GH in fractions #2 and #3. Accordingly these were subjected to extensive bioassay. These results, and their comparisons with the immunoassay results, are summarized in 4 panels on pg. 17 and the table on pg. 18. Collectively these data show that:

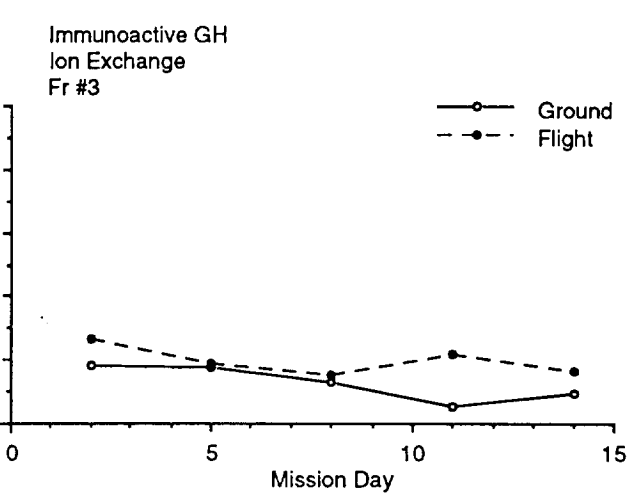
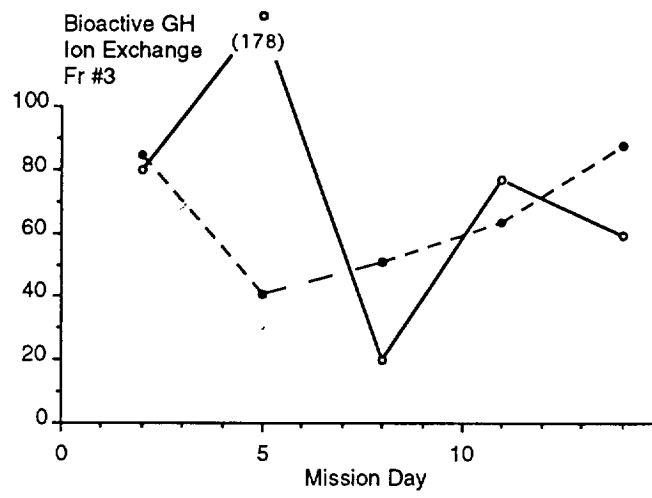
- Early in the culture period (day 5) there is a large increase in bGH output in frs. 2 and 3 from the ground.
- Later into culture (day 14) more bGH is present in these fractions from flight samples.
- The B/I ratios of fraction #2+3 GH are always >1 , usually the ratios of the flight samples are less than the ground samples (pg. 18).



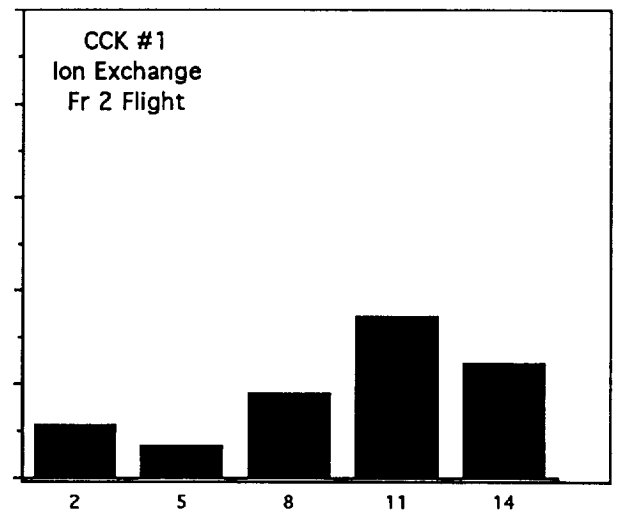
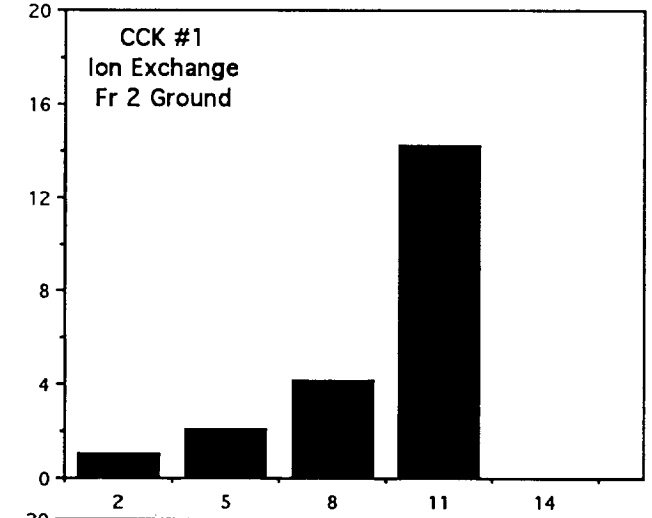
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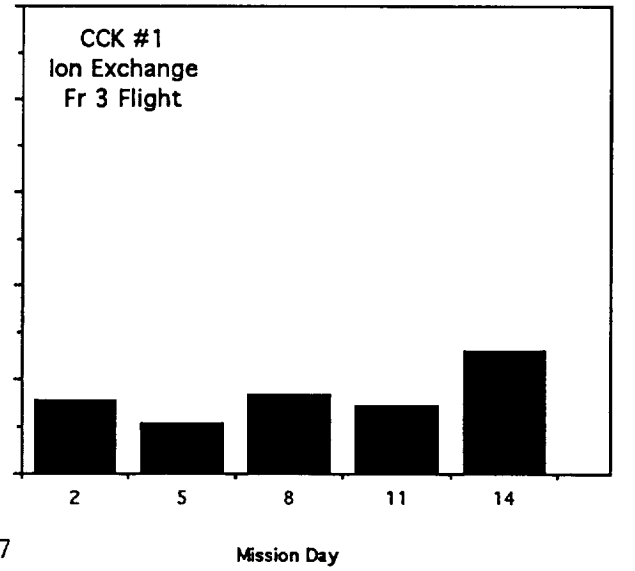
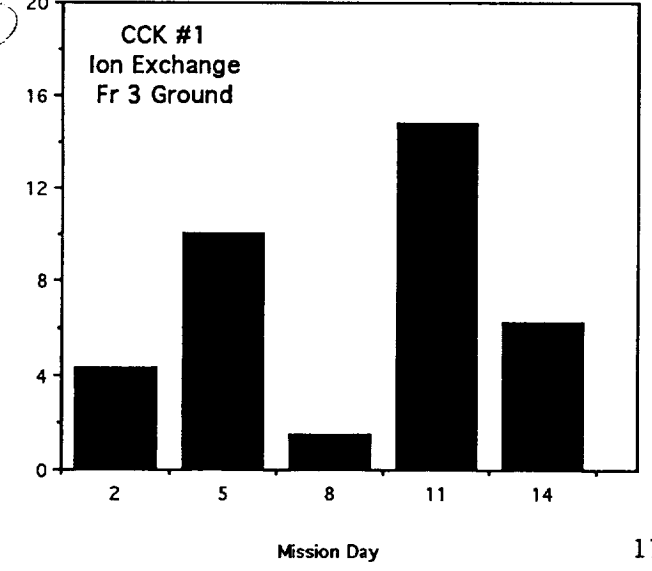
2



3



4



Total Concentrations of bGH and iGH in media from CCK #1
After HPLC Ion Exchange Chromatography:
Combined Fractions #2 and #3

| Day | Bioactive GH ($\mu\text{g/ml}$) | | | Immunoreactive GH ($\mu\text{g/ml}$) | | |
|-----|-----------------------------------|--------|------------------|--|--------|------------------|
| | Flight | Ground | Flight Ground | Flight | Ground | Flight Ground |
| 2 | 137 | 114 | 1.20 | 51 | 52 | 0.98 |
| 8 | 86 | 58 | 1.48 | 25 | 22 | 1.13 |
| 11 | 161 | 143 | 1.12 | 36 | 10 | 3.6 |
| 14 | 144 | 59 | 2.44 | 28 | 13 | 2.2 |

B/I Ratio of GH Contained in Combined HPLC Fractions #2 and #3

| Day | Flight | Ground |
|-----|--------|--------|
| 2 | 2.68 | 2.18 |
| 8 | 3.45 | 2.63 |
| 11 | 4.47 | 14.3 |
| 14 | 5.14 | 4.54 |

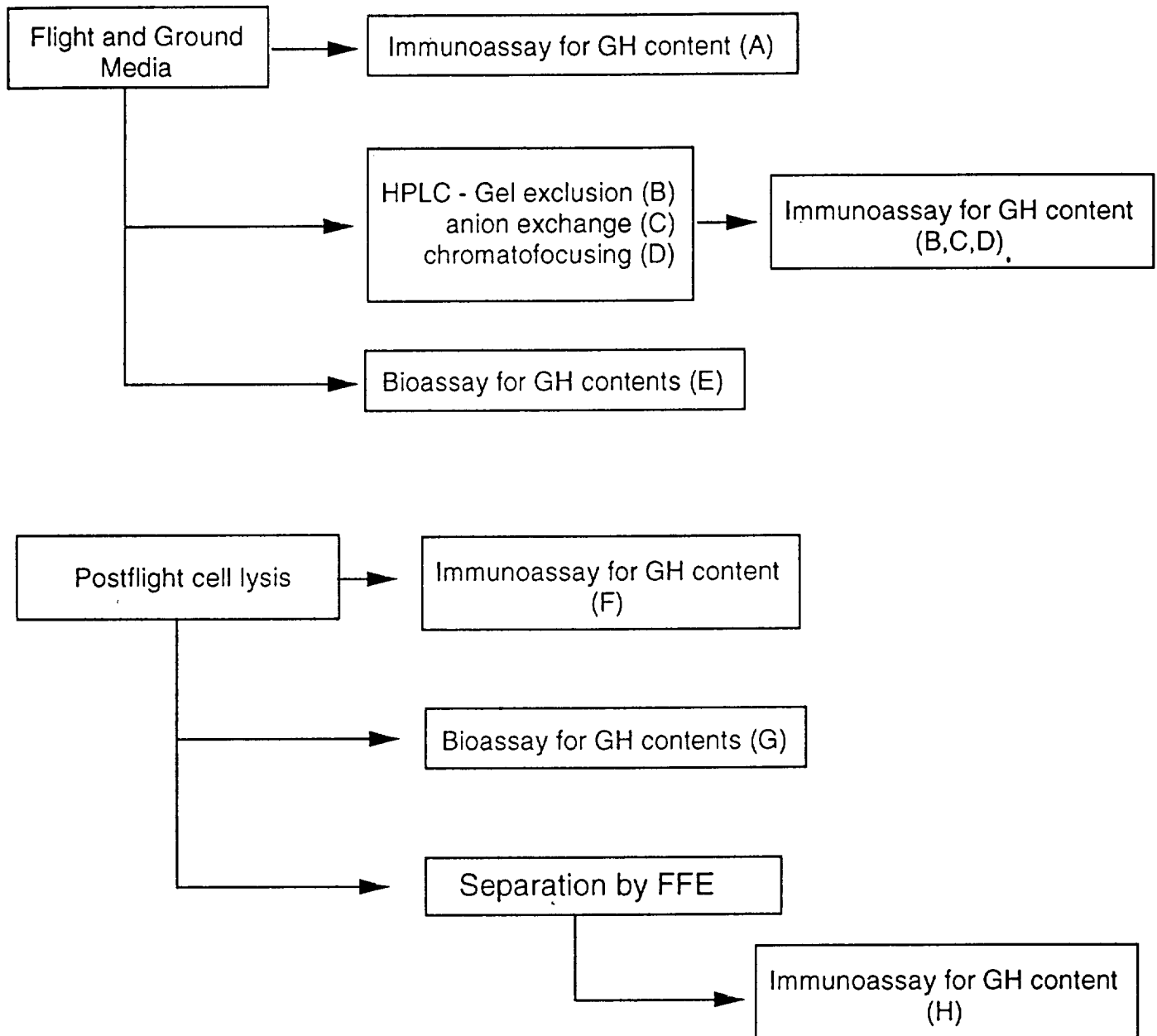
III. CCK #2

CCK #2

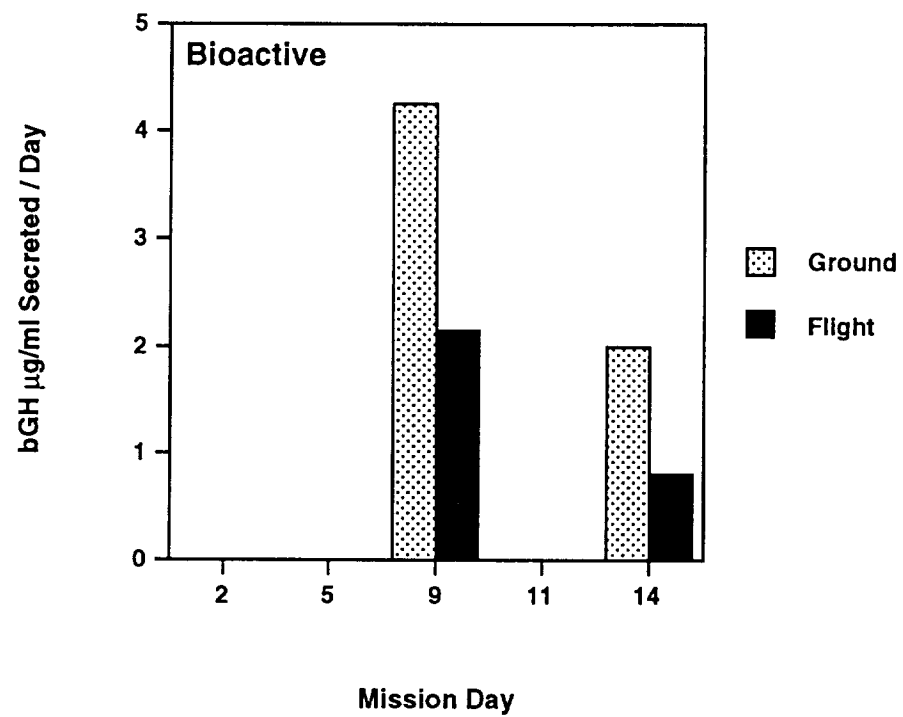
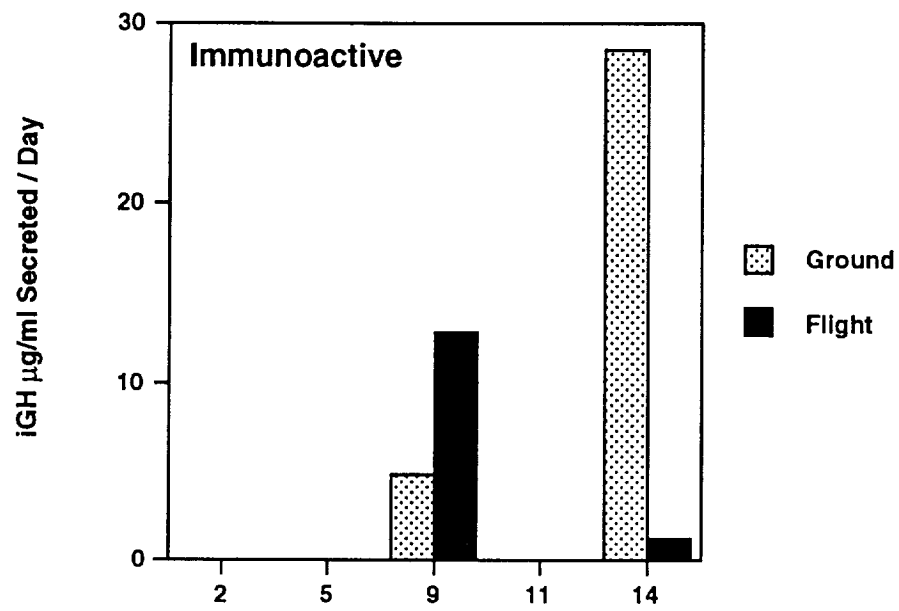
- Because live cells were present in CCK #2 after spaceflight and because no microbial contamination was present on landing, it is obvious that the trypsinization of cells from the CCK and their reinsertion back into the CCK (glovebox operations) were done successfully by the Payload Specialist in microgravity.
- Media changes on day 9 again show that more immunoreactive GH is released from flight cells than ground (compare pg. 4, CCK #2 vs. Pg. 6, CCK #1).
- After cell trypsinization and reinsertion back into CCK #2, ground cells continued to release iGH; however secretion of iGH from flight CCK #2 was greatly reduced (pg. 4). At this point we have no explanation for this result. Release of bGH was lower from flight CCK #2.
- Analysis of mission day 9 media by HPLC sizing chromatography indicated that apparent molecular weights of iGH between ground and flight were not affected, but much more monomeric GH was in the flight media sample (pg. 5).
- Analysis of mission day 14 media by HPLC sizing chromatography shows that while the size profile of the iGH from the ground sample was similar to that of day 9 sample (cf pgs. 5 vs.6), the size profile of the iGH molecules in the flight sample changed. Thus, more high molecular weight material is present in this sample (pg. 6, insert), even though the amount is very much less.
- Osmotic lysis of cells recovered from CCK #2 after landing again shows a difference in apparent sizes (pg. 7) and charge (pg. 8) of iGH.

| Proposed | Actual | % of objective achieved |
|---|---|-------------------------|
| CCK #2 | | |
| • Astronaut takes photomicrographs of cells on MD 5 | Photomicrographs taken, one roll accidentally exposed on orbit, rest of photo's not useable | 2% |
| • Media removed and stored on MD 5 | Media removed and stored on MD 8 | 100% |
| • Cells removed from CCK by trypsin on MD 5 | Cell removed by trypsin on MD 8 (glovebox) | 100% |
| • Trypsinized cells on flight FFE | Not done, hardware malfunction and crew time limitations | 0% |
| • Separated cells into culture. | Trypsinized cells back into CCK for continued culture. | 20% |
| • Separated cells extracted on landing | Recultured cells lysed on landing because volume of lysate from CCK 3 was limiting. Science required more sample for μg effects on intracellular events. | 25% |
| • Synchronous ground operations. | Ground ops performed within 2 hours of flight operations. | 100% |

CCK #2 Growth Hormone Analysis

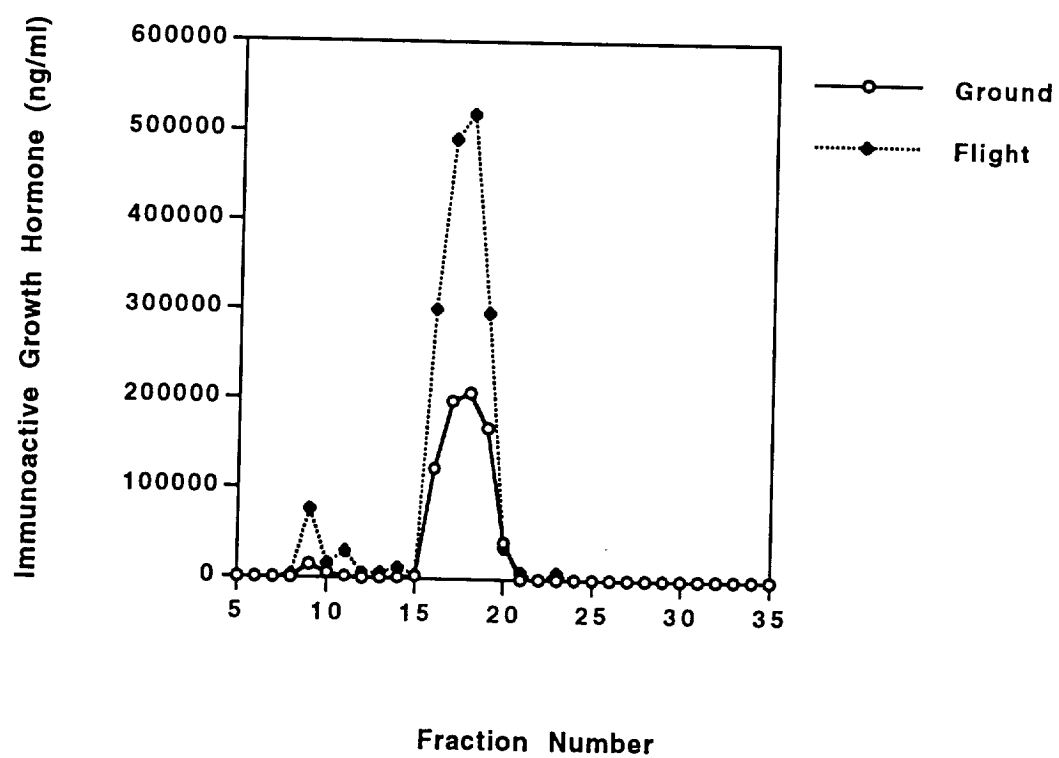


CCK 2 Media Recovered

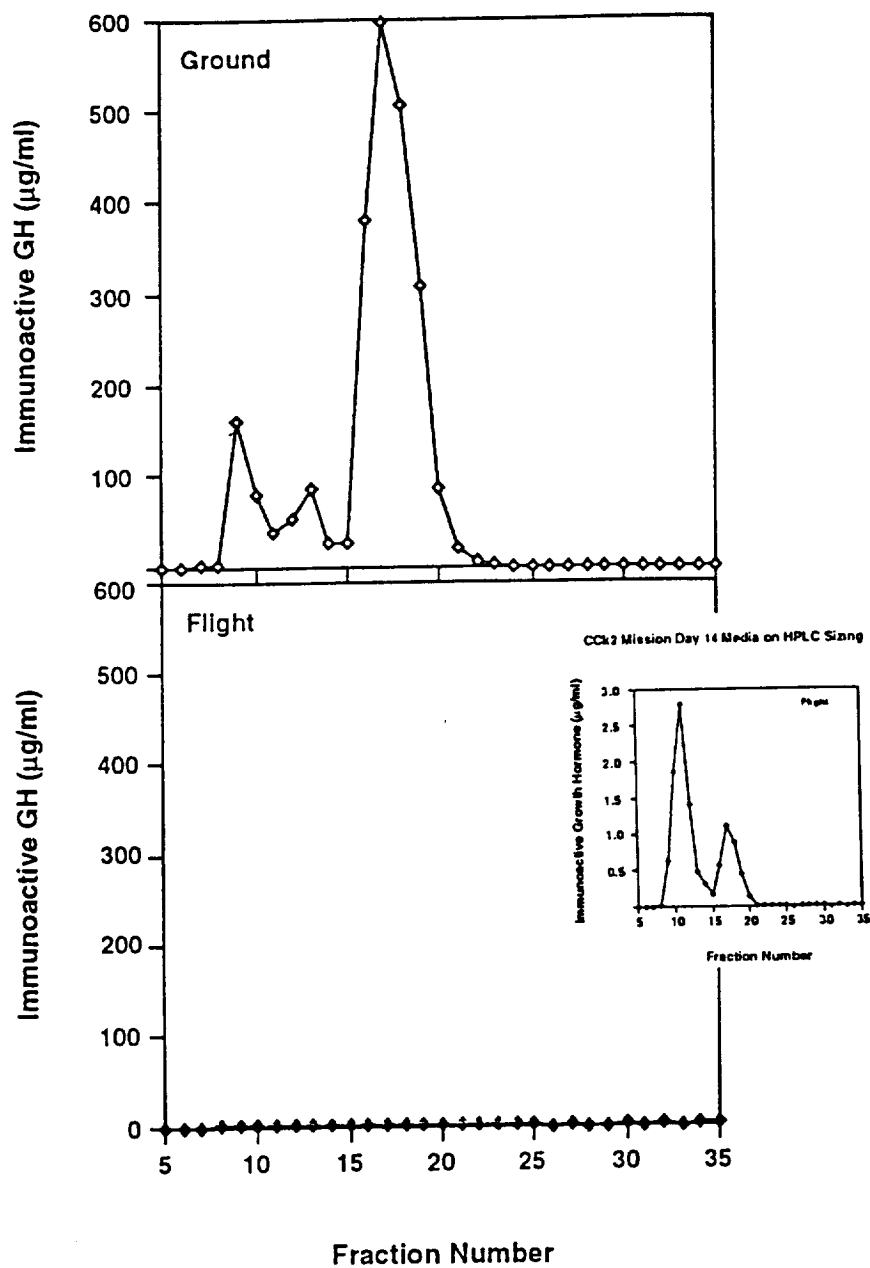


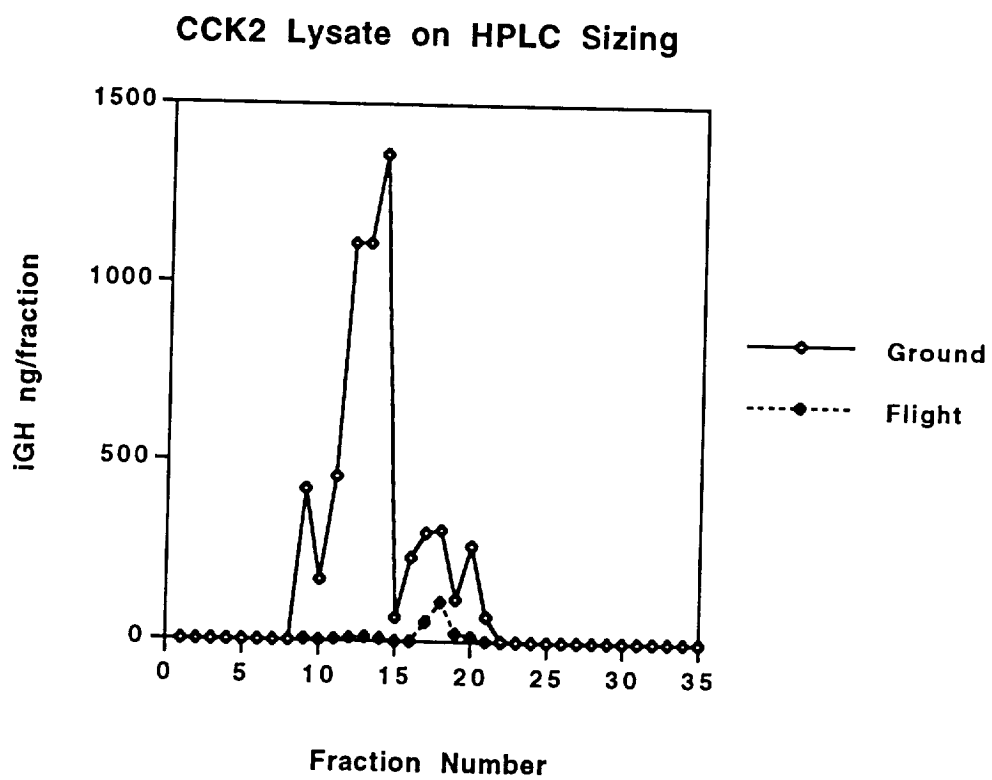
***Note Scale Difference**

CCK 2 Mission Day 9 Media on HPLC Sizing

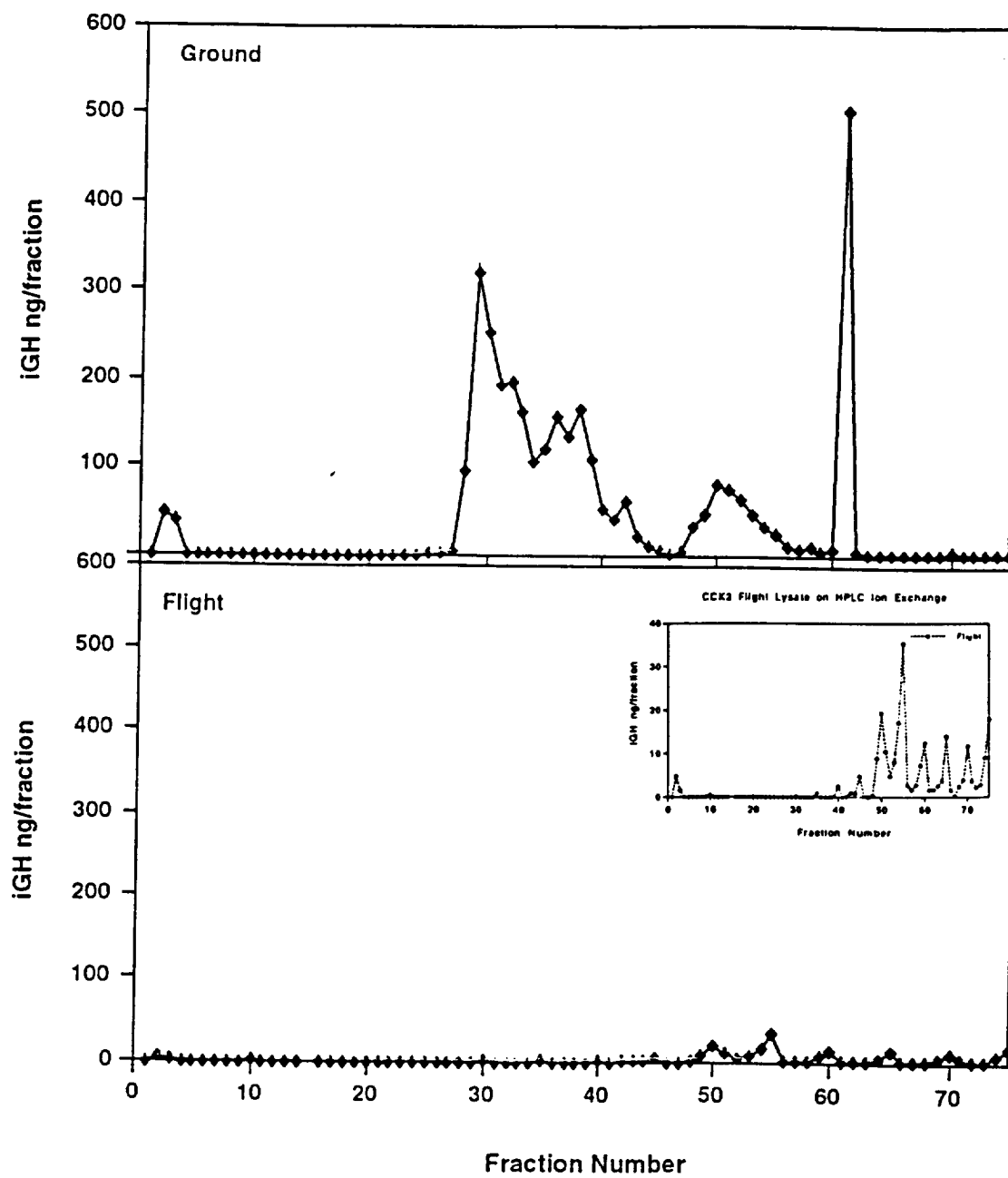


CCK2 Mission Day 14 Media on HPLC Sizing





CCK2 Lysate on HPLC Ion Exchange



Summary of CCK #2. Results to Date: The Bottom line and Tentative Conclusions.

- The astronaut was highly efficient, effective and successful in performing the many complex manipulations that were required on several pieces of hardware.
- After trypsinization, GH cell behavior appears to be quite different in space than on the ground. These differences are most evident in:
 - a) the amount of iGH released into the culture medium
 - b) the apparent size of the released iGH and
 - c) the apparent size and charge of the iGH retained within the cells.

IV. CCK #3

CCK #3

The reader will understand that (a) electrophoresis of pituitary cells and pituitary subcellular particles in microgravity was the primary goal of this experiment and (b) that the FFEU hardware failed on the IML-2 mission. The reader also knows that a major percentage of the actual experiment time necessarily had to be sacrificed for crew in-flight maintenance procedures. In spite of these severe limitations, one electrophoresis trial on the subcellular particles prepared from CCK #3 was attempted in microgravity. The reader may appreciate that the PI and his team (including NASDA personnel) made quick, real time decisions with regard to selection of the 30 tubes for collection knowing full well that some exit ports might be blocked by air bubbles in the separation chamber. The following descriptions of the data collected on these 30 fractions thus far offer the HINT that some fractionation may have occurred in microgravity.

- Pg. 3 - suggests that apart from the actual FFE run, many experimental objectives dealing with CCK #3 were achieved.
- Pg. 4 - downlinked UV absorption data during electrophoresis run (top panel) led the PI to select frs. 8-13 and frs. 24-47 for collection. On return to Penn State some fractions contained close to their nominal 2.5 ml, while many did not (bottom panel). Is this evidence for bubble blockage??
- Pg. 6 - The concentration of iGH in the culture media of CCK #3 shows that more immunoactive hormone is released from flight cells than ground. This result is entirely consistent (and very similar to) that found in culture media from the CCK #2 sample. However, the result with bGH is different between CCR #2 and #3. We do not know why.
- Pg. 7 - Details of FFE operating conditions demonstrate one advantage of microgravity processing by FFE.
- Pg. 8 - distribution of iGH in FFE fractions. Greater bandspread and more GH peaks suggest better separation in microgravity.
- Pg. 9 - Western blots of iGH molecules contained in different FFE fractions. These are non-reducing SDS-PAGE gels; all bands represent different molecular weight forms of GH. These are the result of aggregation/splicing variants. Some evidence for differences in high MW variants in different flight fractions is seen (arrows). Densitometry will be done on these blots to identify and quantify these differences.
- Pg. 10a,b,c, - HPLC gel sizing chromatograms (OD 280) of pituitary cell lysates prepared in space (10a) or on earth (10b). The difference spectra

(10c) indicate some significant protein profile differences between ground and flight.

- Pg. 10d-k - HPLC sizing chromatograms of proteins contained in FFE fractions prepared in space or on earth. Note that some protein material present in the starting sample (e.g. that at retention time 16.6 min, pg. 10a,b) fails to appear after the FFE run.
- Pg. 10L - Superimposed protein profiles of flight only FFE fractions after HPLC. Clear-cut differences in these profiles support the thesis that separation on the FFEU was occurring in microgravity.
- Pg. 10m-s - HPLC ion exchange chromatographic profiles of proteins extracted from FFE fractions (ground and flight). These have not been evaluated for additional evidence of separation.

| Proposed | Actual | % of objective achieved |
|---|---|-------------------------|
| CCK #3 | | |
| • Media removed and stored on MD 5 | Media removed and stored on MD 9 | 100% |
| • Cell lysis / granule concentration | Accomplished, volume after concentration unknown. † | 90% |
| • Lysate on FFE in μg and collection of 30 fractions with 5 mls each | Lysate on FFE and collection of 30 fractions in microgravity. †† | 30% |
| • Lysate fractions frozen at -20°C in microgravity. | Lysate fractions frozen at -20°C in microgravity. | 100% |
| • Perform synchronous ground operations. | Ground operations performed within 15 minutes of flight operations. | 100% |

† Estimated volume on basis of recovered fluid.

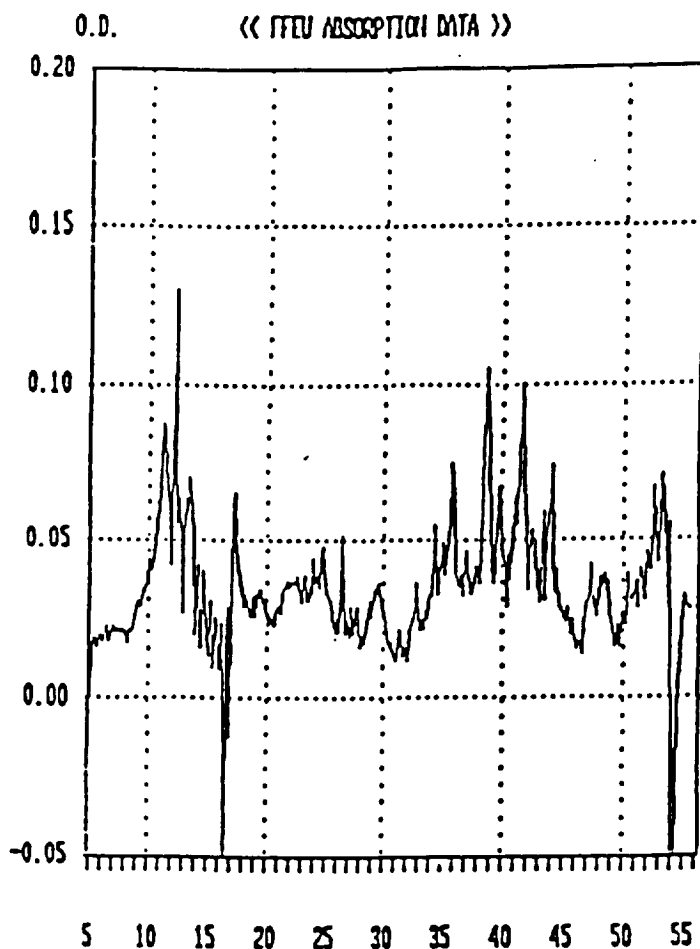
a) 28 minutes of FFE run time (8' no field/20' @ 25 v/cm) \approx 1 ml.

b) Volume in FFE sample syringe \approx 1 ml

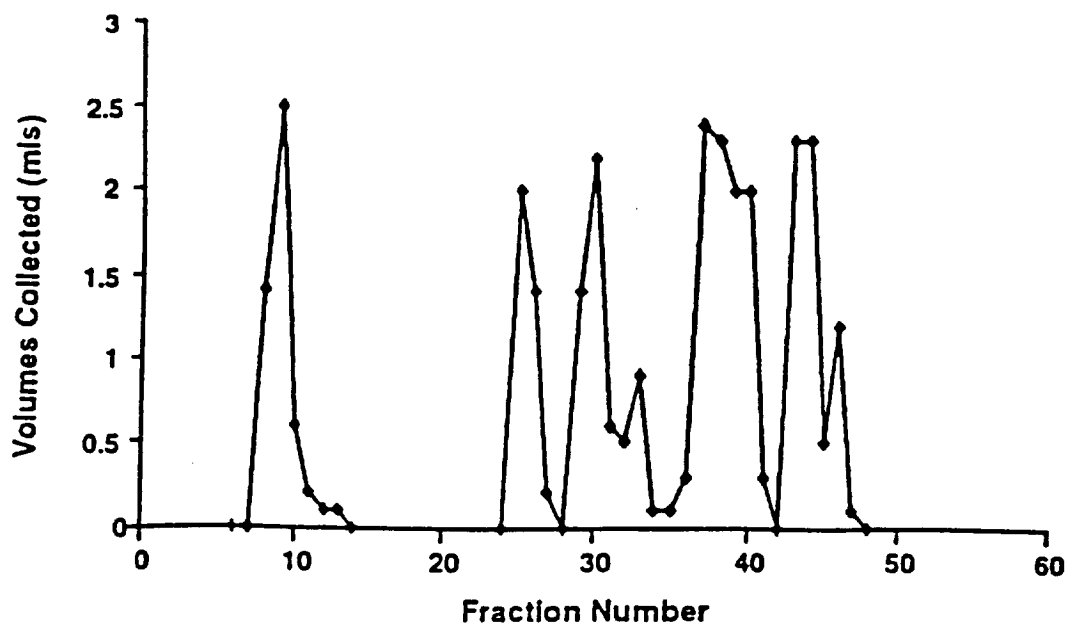
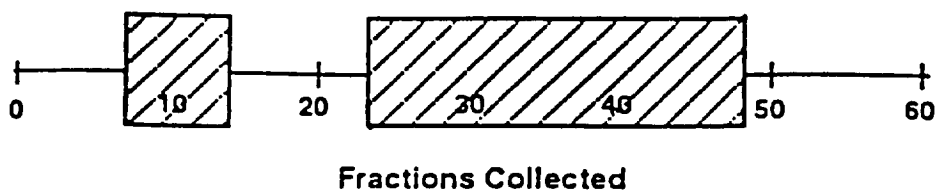
c) Volume remaining in CCK#3 which did not get concentrated \approx 2.5 ml.

†† Bubbles in separation chamber at time of run. Downlink optical density profile (see page22) suggests sample flow with and without field. See Fig5. for additional information.

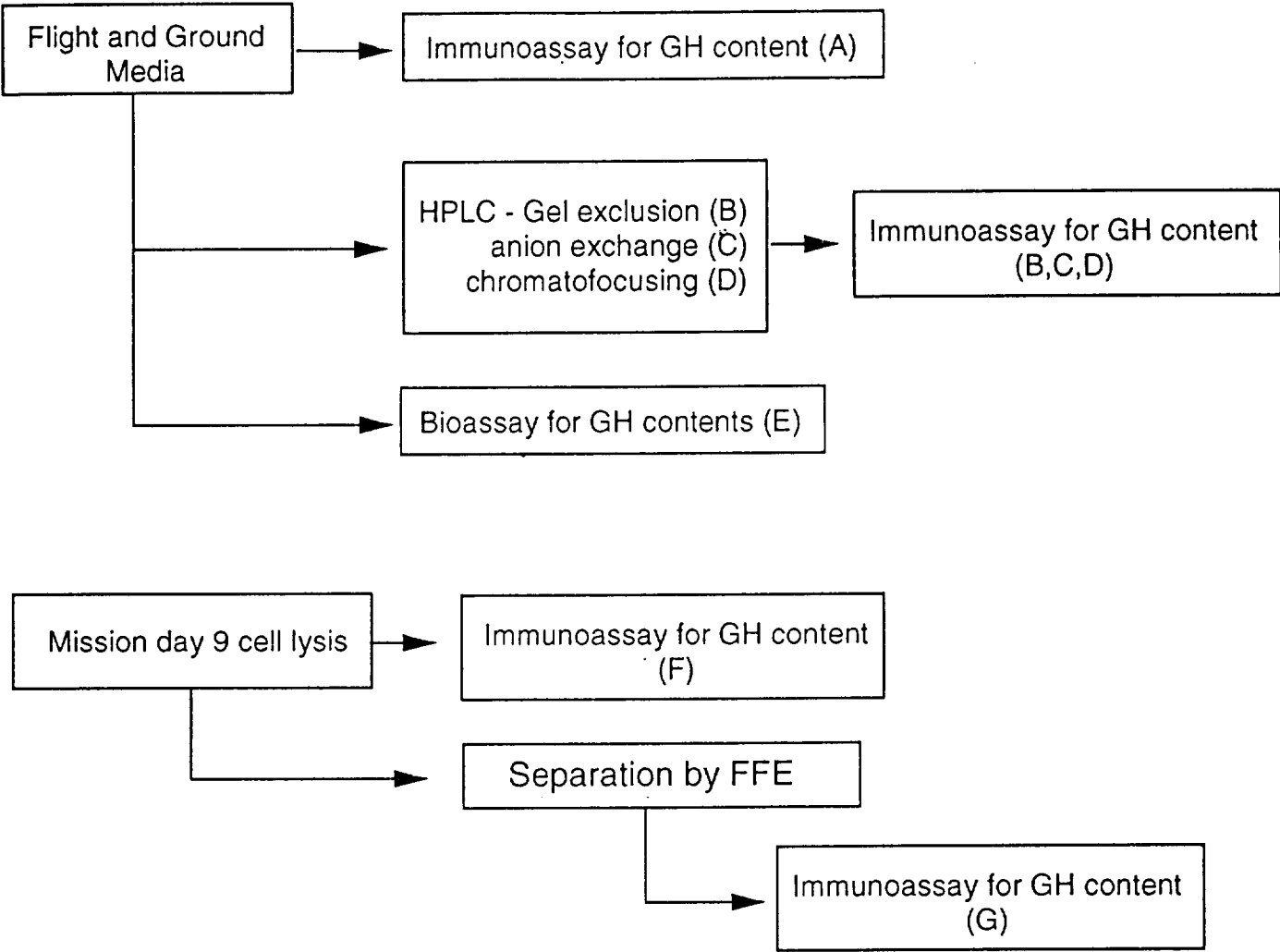
A).



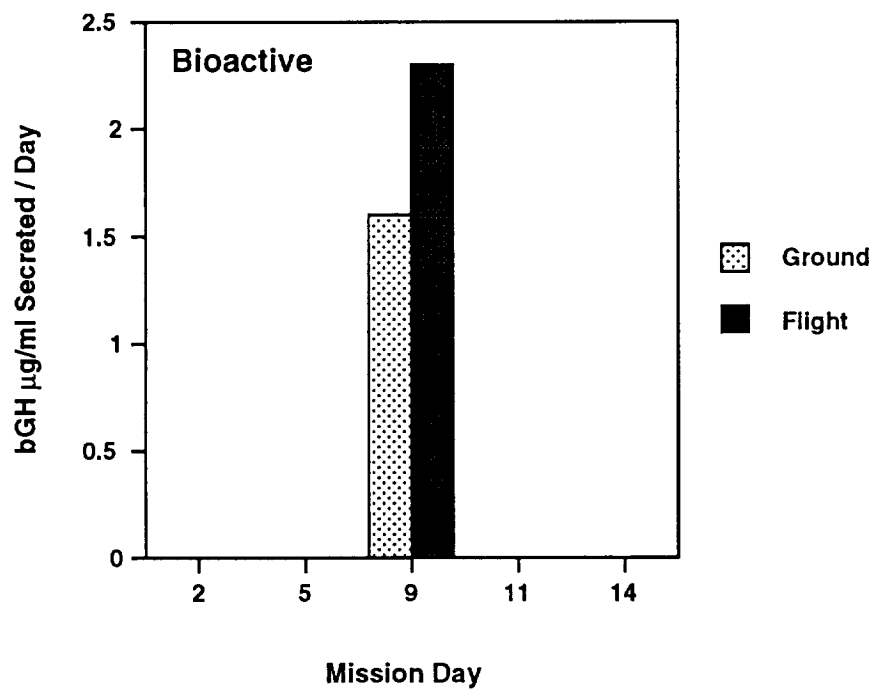
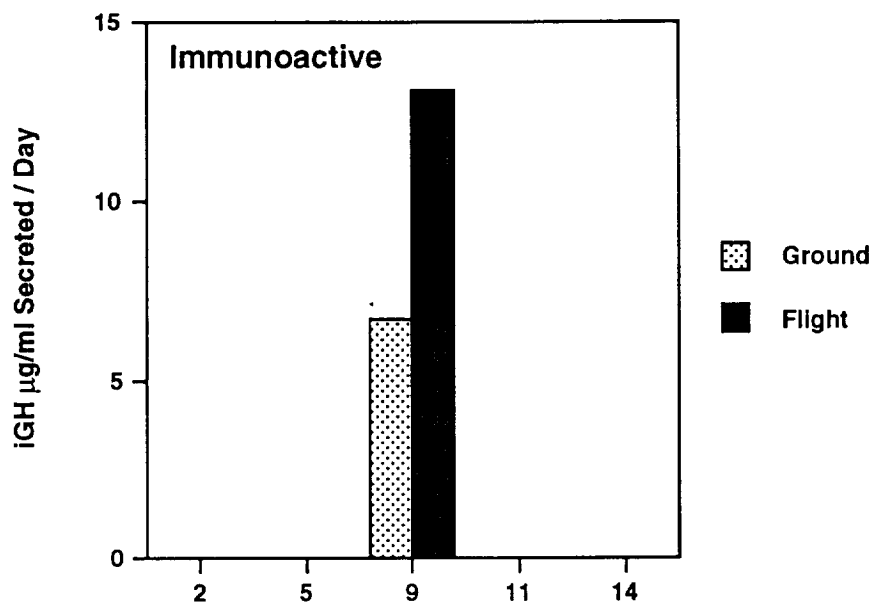
B).



CCK #3 Growth Hormone Analysis



CCK 3 Media Recovered



***Note Scale Difference**

IML-2 CCK-3 Lysate Processing

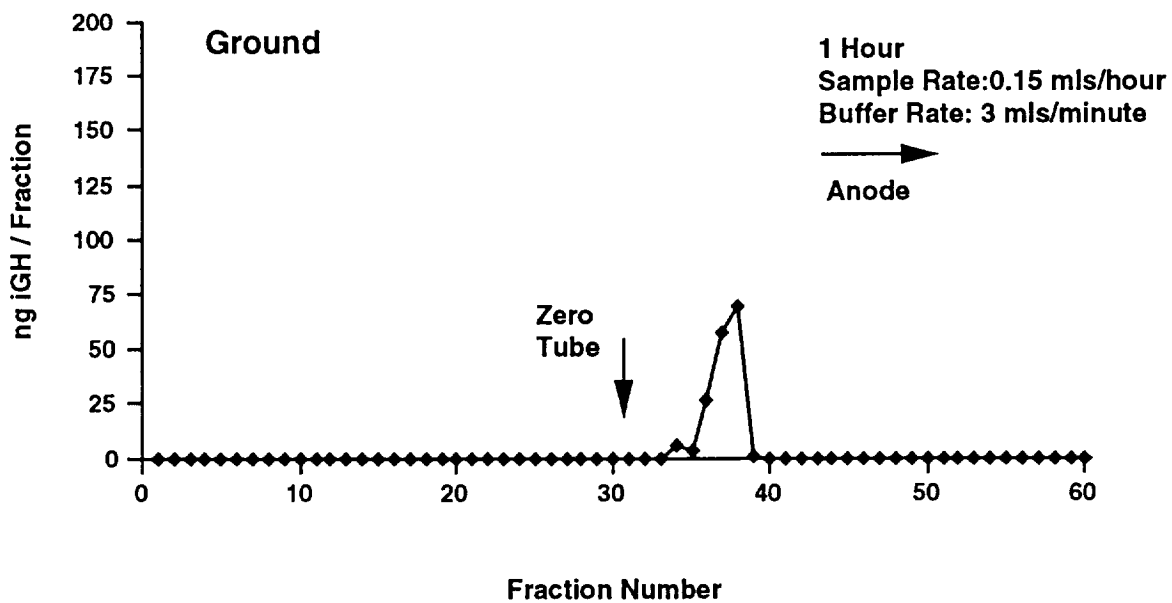
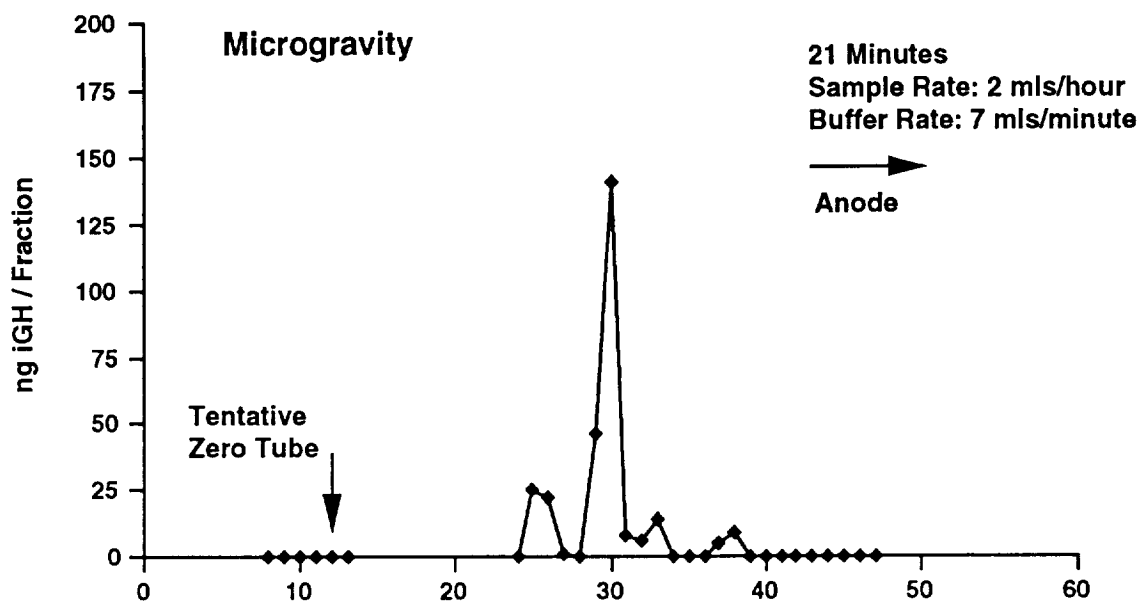
Flight

- 20 minute processing time
- 0.7 ml processed
- 150 ml carrier buffer
- 21.4 x dilution effect**

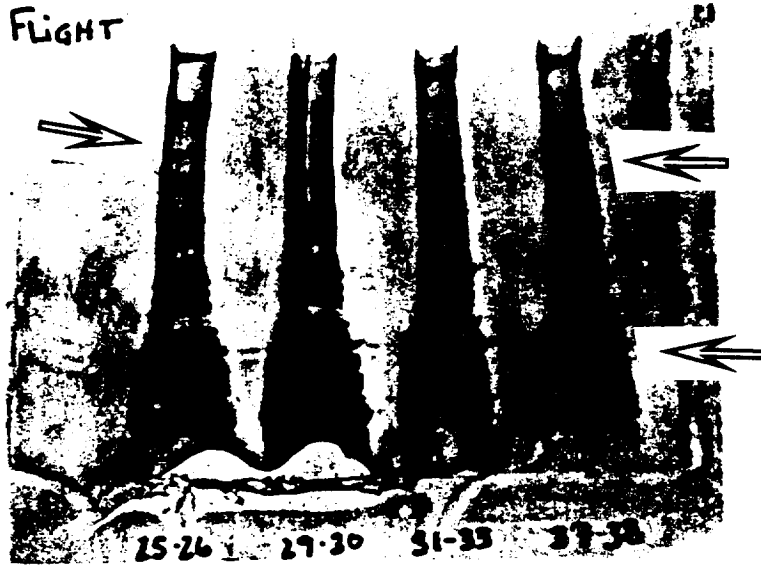
Ground

- 60 minute processing time
- 0.15 ml processed
- 180 ml carrier buffer
- 1200 x dilution effect**

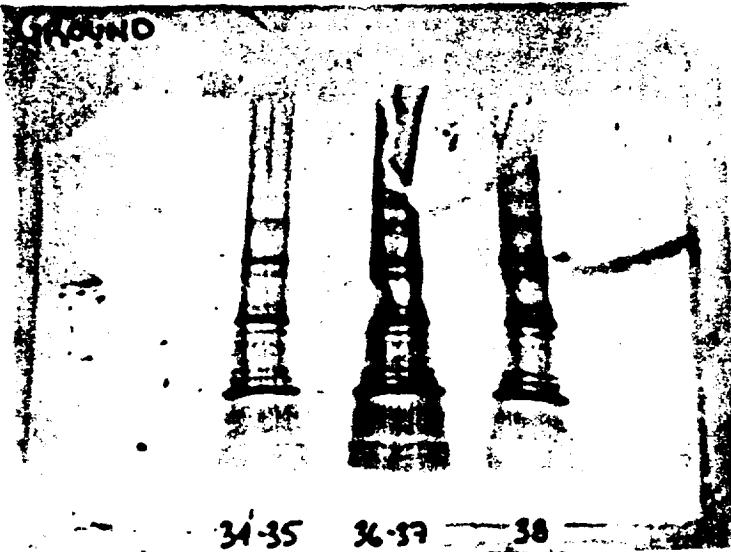
Therefore the flight sample is **56 x** more concentrated than the ground sample



FLIGHT



GROUND



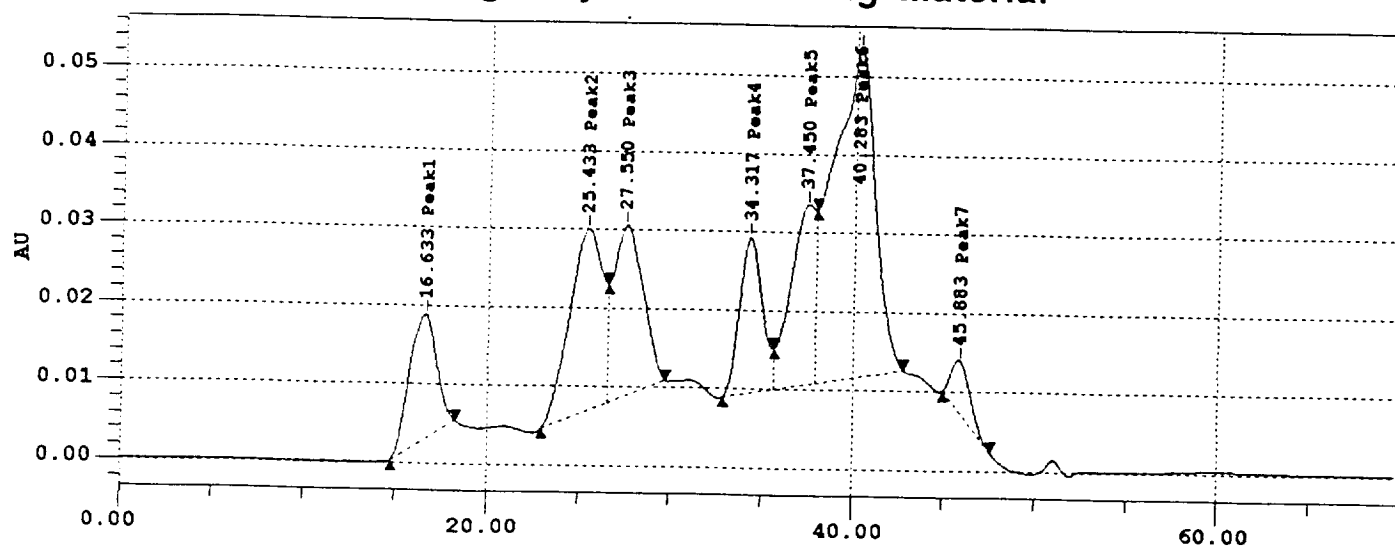
For Sample: CCK3-flight-ly Vial: 4 Inj: 1 Chan: 490 Ch1 Date Processed 02/28/95 08:55 AM

Millennium Sample Information

Project Name: kris
Sample Name: CCK3-flight-ly
Vial: 4
Injection: 1
Channel: 490 Ch1
Date Acquired: 01/26/95 01:01 PM
Scale Factor: 1.00
Acq Meth Set: kris_ms
Processing Method: cck3Fly

Sample Type: Unknown
Volume: 300.00
Run Time: 70.0 min
Date Processed: 02/28/95 08:55 AM
Dilution: 1.00000

Flight Lysate - Starting Material



Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 16.633 | 1643040 | 15850 | | BB |
| 2 | Peak2 | 25.433 | 2918329 | 23183 | | BV |
| 3 | Peak3 | 27.550 | 2484895 | 21707 | | VB |
| 4 | Peak4 | 34.317 | 1718907 | 19845 | | BV |
| 5 | Peak5 | 37.450 | 2108257 | 22953 | | VV |
| 6 | Peak6 | 40.283 | 6864648 | 41906 | | VB |
| 7 | Peak7 | 45.883 | 485699 | 6686 | | BB |

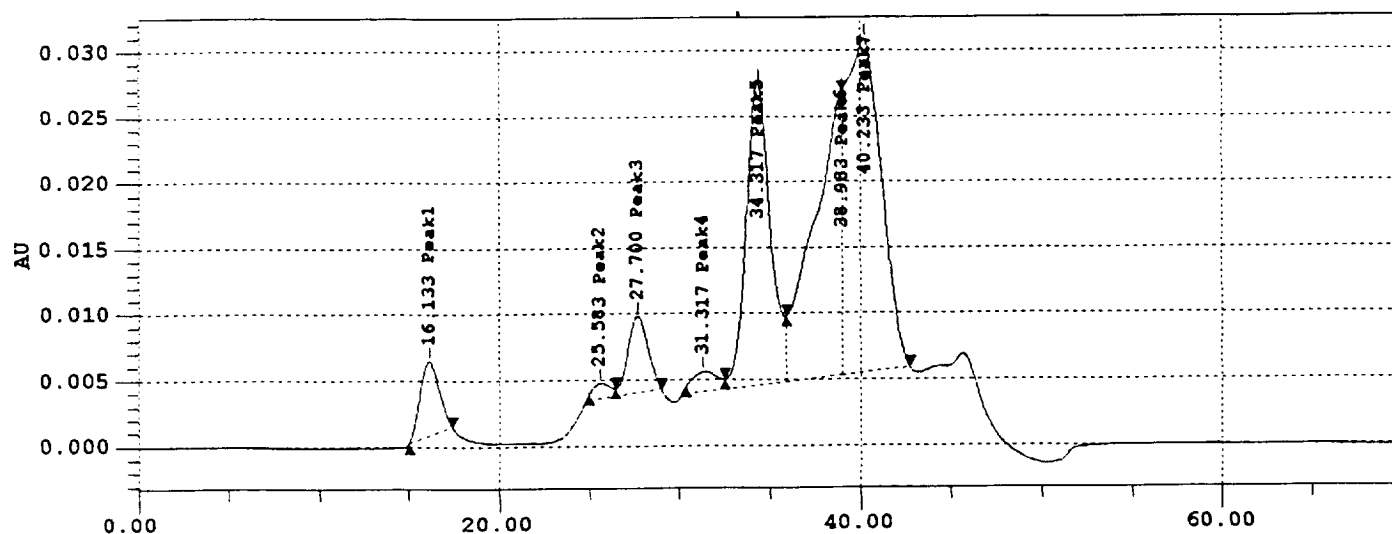
For Sample: CCK3-GR-lysate Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 02/28/95 08:28 AM

Millennium Sample Information

Project Name: kris
 Sample Name: CCK3-GR-lysate
 Vial: 1
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 01/25/95 01:45 PM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: cck3ly

Sample Type: Unknown
 Volume: 300.00
 Run Time: 70.0 min
 Date Processed: 02/28/95 08:28 AM
 Dilution: 1.00000

Ground Lysate - Starting Material

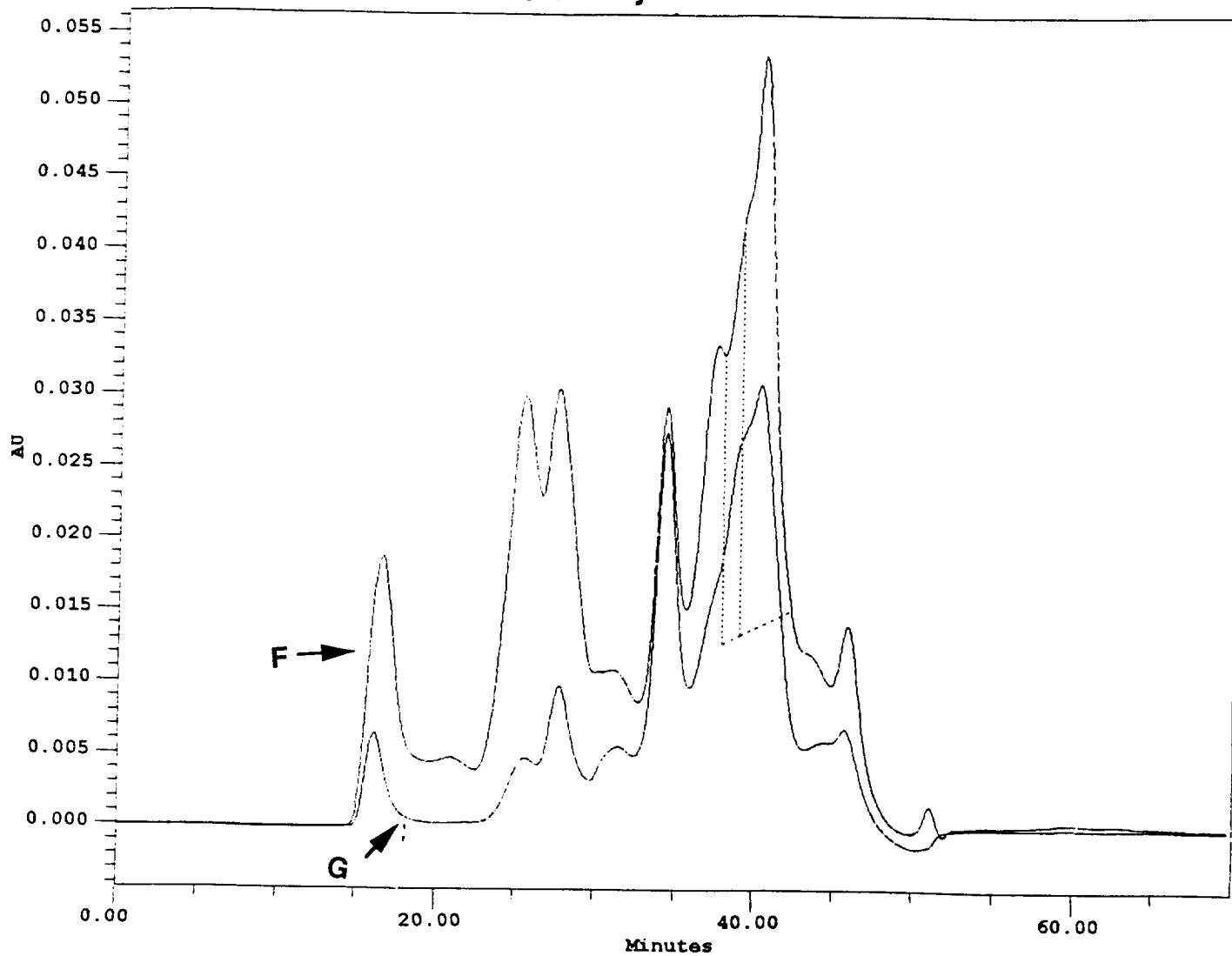


Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 16.133 | 426331 | 5686 | | BB |
| 2 | Peak2 | 25.583 | 84771 | 1262 | | VV |
| 3 | Peak3 | 27.700 | 451661 | 5803 | | VB |
| 4 | Peak4 | 31.317 | 151576 | 1580 | | VV |
| 5 | Peak5 | 34.317 | 2147813 | 22976 | | VV |
| 6 | Peak6 | 38.983 | 2276186 | 21506 | | VV |
| 7 | Peak7 | 40.233 | 3497883 | 25384 | | VB |

Date February 28, 1995

Start Lysate



Samples

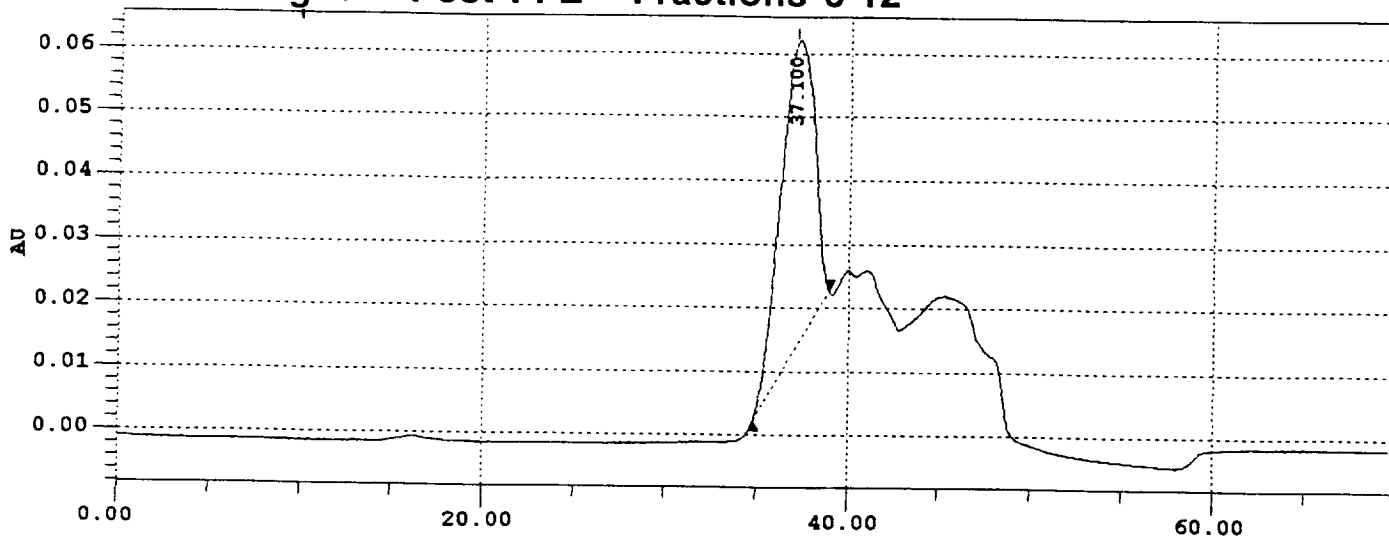
| # | Sample Name | Vial | Inj | Channel | Date Processed |
|---|----------------|------|-----|---------|-------------------|
| 1 | CCK3-flight-ly | 4 | 1 | 490 Ch1 | 01/26/95 02:13 PM |
| 2 | CCK3-GR-lysate | 1 | 1 | 490 Ch1 | 01/25/95 02:57 PM |

For Sample: CCK3-FR8-12F Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 02/28/95 02:22 PM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | CCK3-FR8-12F | Volume: | 300.00 |
| Vial: | 1 | Run Time: | 70.0 min |
| Injection: | 1 | Date Processed: | 02/28/95 02:22 PM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 02/28/95 01:11 PM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |

Flight - Post FFE Fractions 8-12



Peak Results

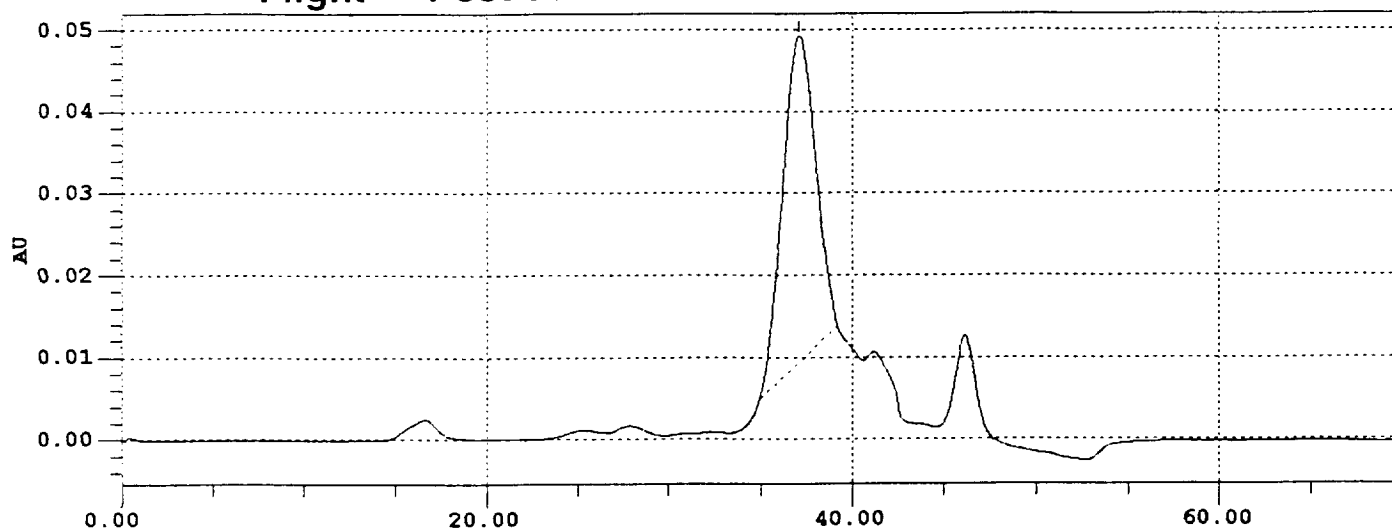
| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |
| 4 | | 37.100 | 6020124 | 48072 | | BB |

For Sample: CCK3-F-fr25-26 Vial: 5 Inj: 1 Chan: 490 Ch1 Date Processed 01/26/95 03:45 PM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | CCK3-F-fr25-26 | Volume: | 300.00 |
| Vial: | 5 | Run Time: | 70.0 min |
| Injection: | 1 | Date Processed: | 01/26/95 03:45 PM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 01/26/95 02:33 PM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |

Flight - Post FFE Fractions 25-26



Peak Results

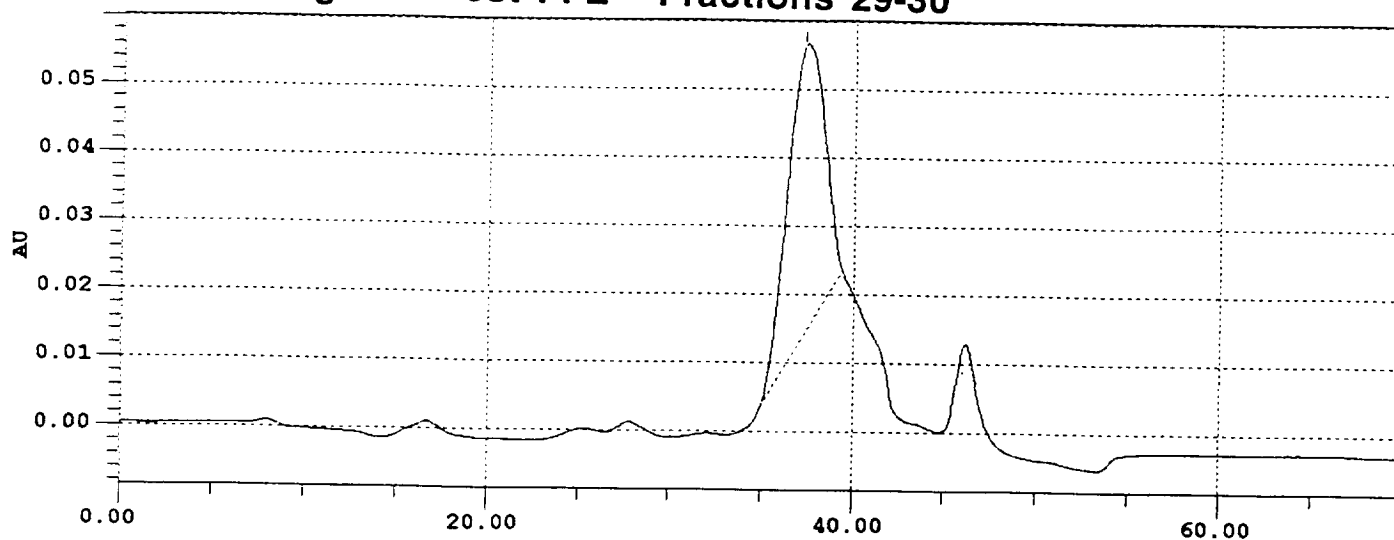
| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |
| 4 | | 37.083 | 5016891 | 39976 | | BB |

For Sample: CCK3-F-fr29-30 Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 01/27/95 08:52 AM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | CCK3-F-fr29-30 | Volume: | 300.00 |
| Vial: | 1 | Run Time: | 70.0 min |
| Injection: | 1 | Date Processed: | 01/27/95 08:52 AM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 01/27/95 07:40 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |

Flight - Post FFE Fractions 29-30



Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|-------------------|------------------|----------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |
| 4 | | 37.267 | 5844754 | 42478 | | BB |

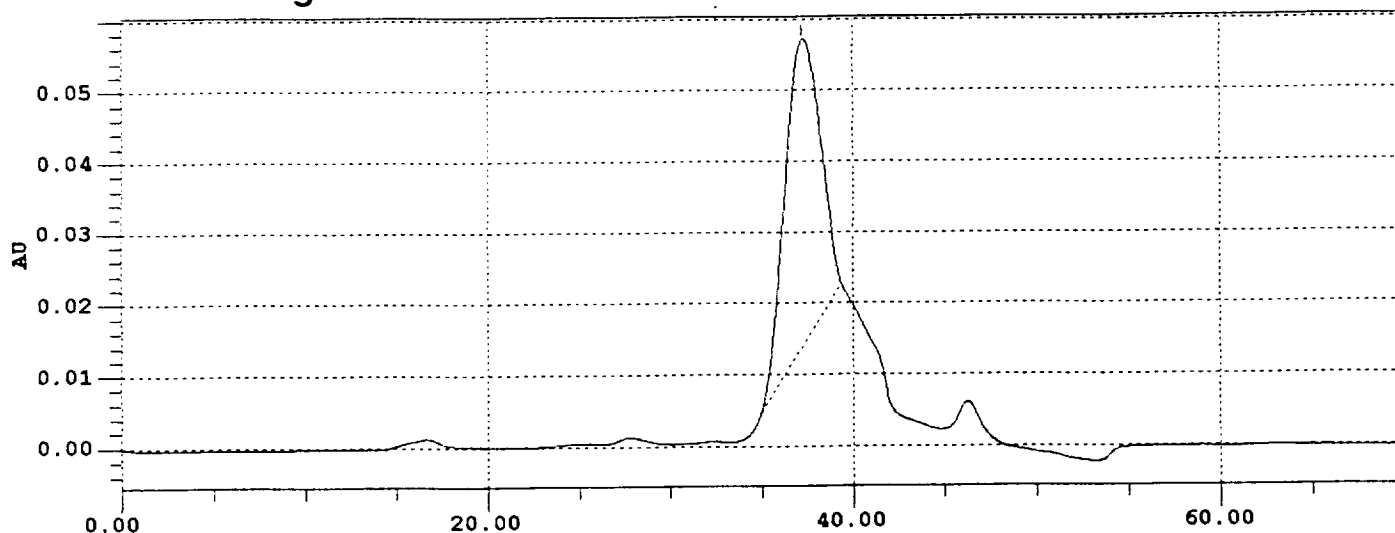
For Sample: CCK3-F-fr31-33 Vial: 2 Inj: 1 Chan: 490 Ch1 Date Processed 01/27/95 10:10 AM

Millennium Sample Information

Project Name: kris
 Sample Name: CCK3-F-fr31-33
 Vial: 2
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 01/27/95 08:58 AM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: kris_pm

Sample Type: Unknown
 Volume: 300.00
 Run Time: 70.0 min
 Date Processed: 01/27/95 10:10 AM
 Dilution: 1.00000

Flight - Post FFE Fractions 31-33



Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |
| 4 | | 37.233 | 5848591 | 43213 | | BB |

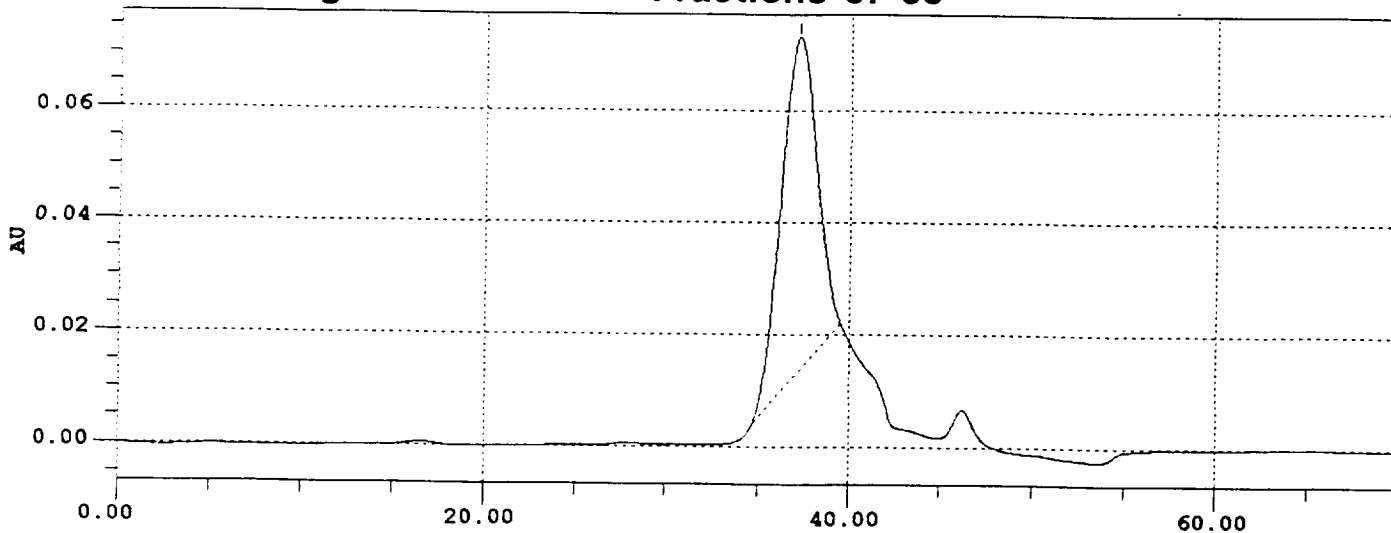
For Sample: CCK3-F-fr37-38 Vial: 3 Inj: 1 Chan: 490 Ch1 Date Processed 01/27/95 11:31 AM

Millennium Sample Information

Project Name: kris
 Sample Name: CCK3-F-fr37-38
 Vial: 3
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 01/27/95 10:20 AM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: kris_pm

Sample Type: Unknown
 Volume: 300.00
 Run Time: 70.0 min
 Date Processed: 01/27/95 11:31 AM
 Dilution: 1.00000

Flight - Post FFE Fractions 37-38



Peak Results

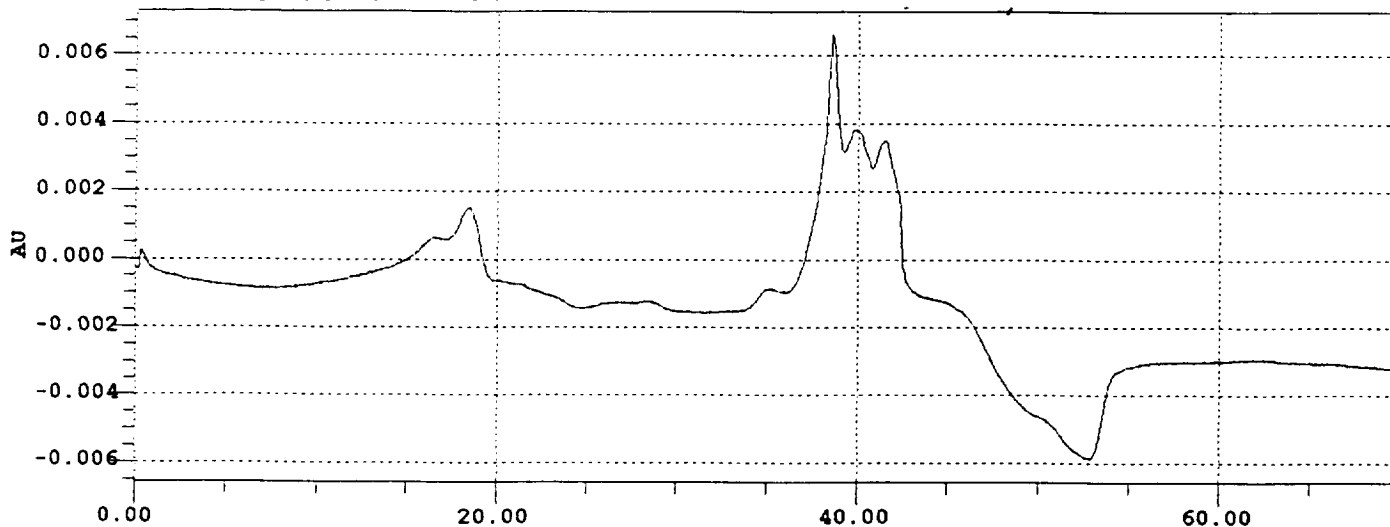
| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |
| 4 | | 37.150 | 8171734 | 60268 | | BB |

For Sample: CCK3-G-fr34-35 Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 01/26/95 09:18 AM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | CCK3-G-fr34-35 | Volume: | 300.00 |
| Vial: | 1 | Run Time: | 70.0 min |
| Injection: | 1 | Date Processed: | 01/26/95 09:18 AM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 01/26/95 08:06 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |

Ground - Post FFE Fractions 34-35



Peak Results

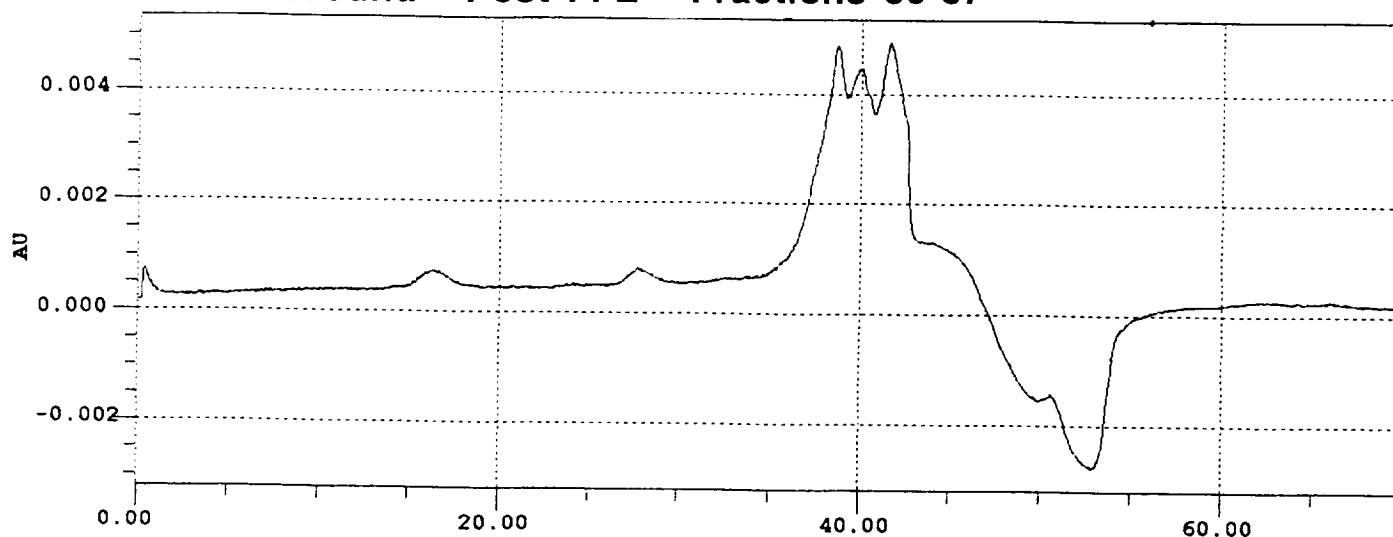
| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|-------------------|------------------|----------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

For Sample: CCK3-G-fr36-37 Vial: 2 Inj: 1 Chan: 490 Ch1 Date Processed 01/26/95 10:48 AM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | CCK3-G-fr36-37 | Volume: | 300.00 |
| Vial: | 2 | Run Time: | 70.0 min |
| Injection: | 1 | Date Processed: | 01/26/95 10:48 AM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 01/26/95 09:36 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |

Ground - Post FFE Fractions 36-37



Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|-------------------|------------------|----------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

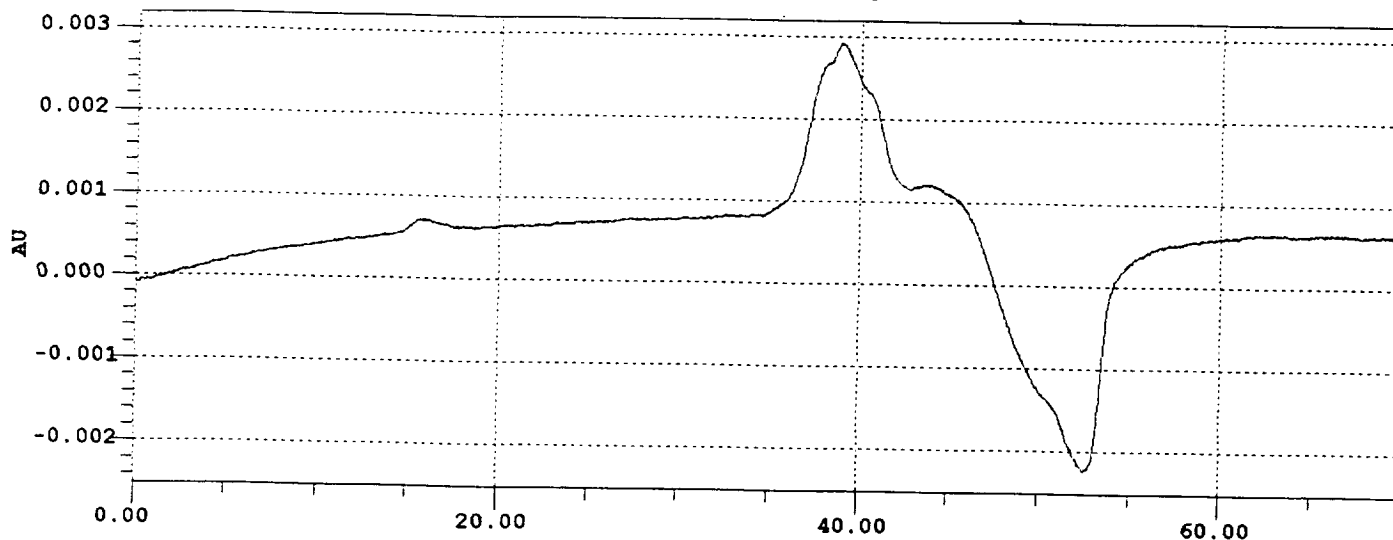
For Sample: CCK3-G-fr38 Vial: 3 Inj: 1 Chan: 490 Ch1 Date Processed 01/26/95 12:08 PM

Millennium Sample Information

Project Name: kris
 Sample Name: CCK3-G-fr38
 Vial: 3
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 01/26/95 10:56 AM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: kris_pm

Sample Type: Unknown
 Volume: 300.00
 Run Time: 70.0 min
 Date Processed: 01/26/95 12:08 PM
 Dilution: 1.00000

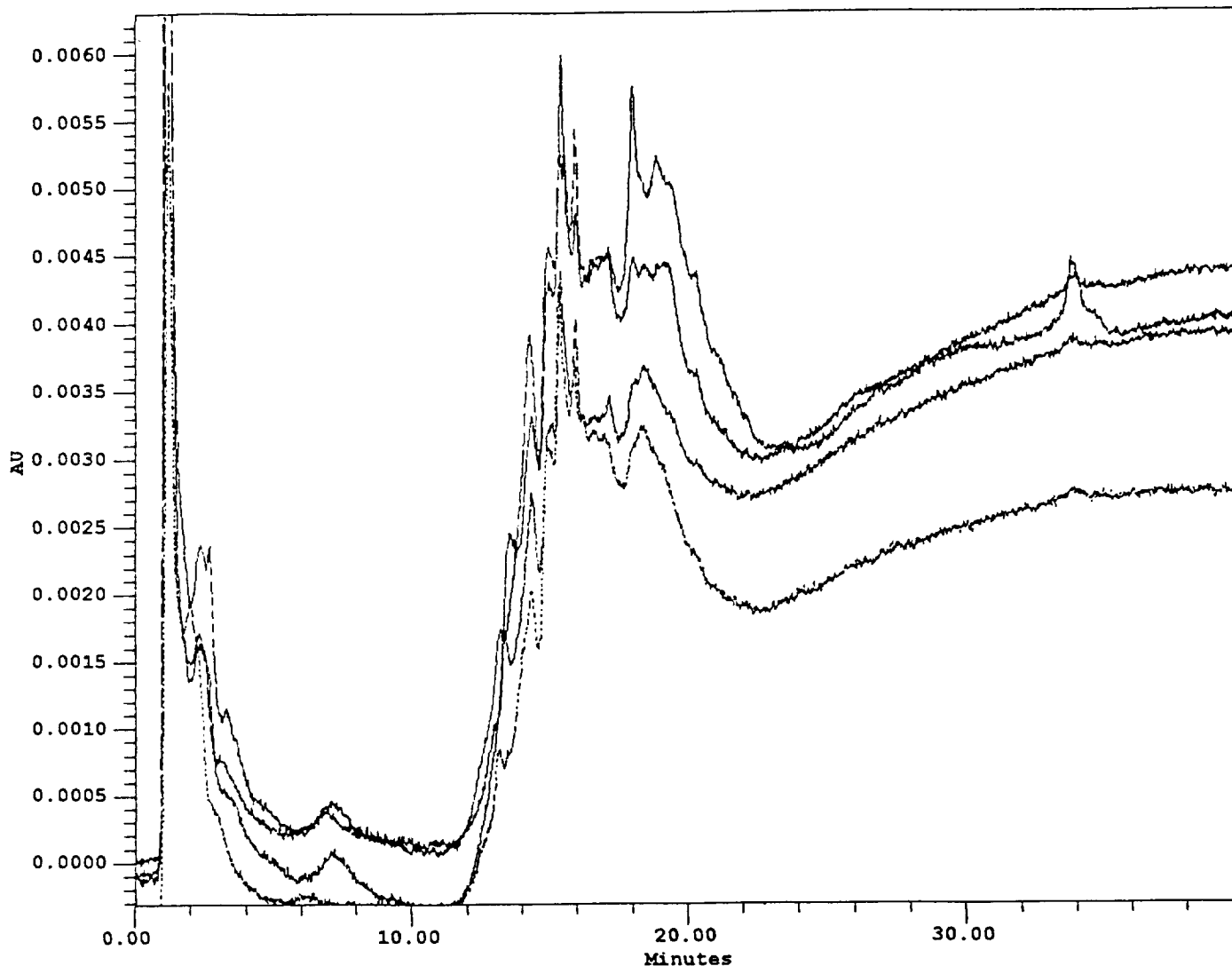
Ground - Post FFE Fraction 38



Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|-------------------|------------------|----------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

Comparisons of OD 280 Profiles of Proteins Contained in Different FFE Fractions (Flight Only)



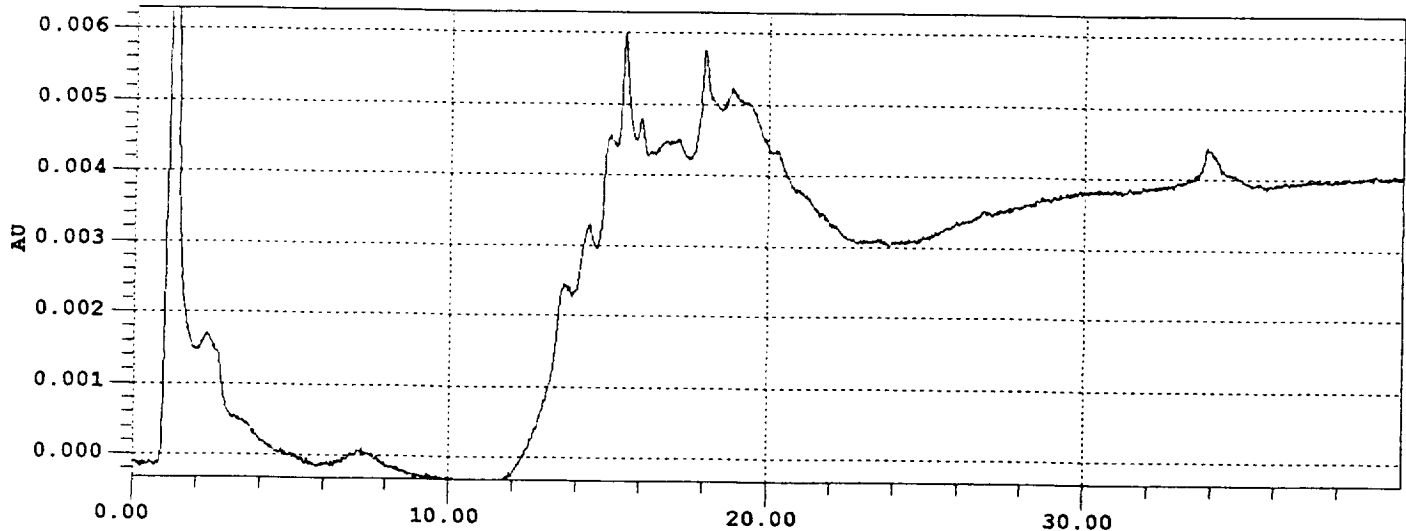
Samples

| # | Sample Name | Vial | Inj | Channel | Date Processed |
|---|----------------|------|-----|---------|-------------------|
| 1 | cck3fr25-26Fio | 1 | 2 | 490 Ch1 | 03/02/95 08:20 AM |
| 2 | cck3fr29-30Fio | 2 | 1 | 490 Ch1 | 03/02/95 09:16 AM |
| 3 | cck3fr31-33Fio | 3 | 1 | 490 Ch1 | 03/02/95 10:11 AM |
| 4 | cck3fr36-37Fio | 4 | 1 | 490 Ch1 | 03/02/95 11:02 AM |

For Sample: cck3fr25-26Fio Vial: 1 Inj: 2 Chan: 490 Ch1 Date Processed 03/02/95 08:20 AM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | cck3fr25-26Fio | Volume: | 200.00 |
| Vial: | 1 | Run Time: | 45.0 min |
| Injection: | 2 | Date Processed: | 03/02/95 08:20 AM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 03/02/95 07:34 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |



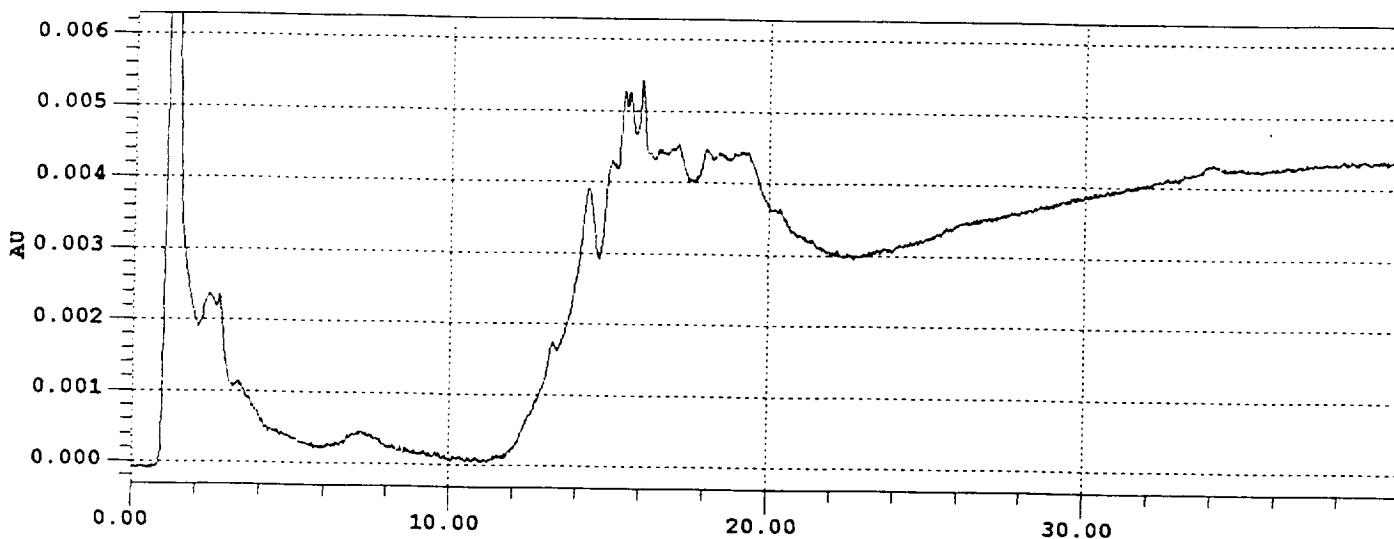
Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

For Sample: cck3fr29-30F10 Vial: 2 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 04:40 PM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | cck3fr29-30F10 | Volume: | 200.00 |
| Vial: | 2 | Run Time: | 45.0 min |
| Injection: | 1 | Date Processed: | 03/02/95 04:40 PM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 03/02/95 08:30 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |



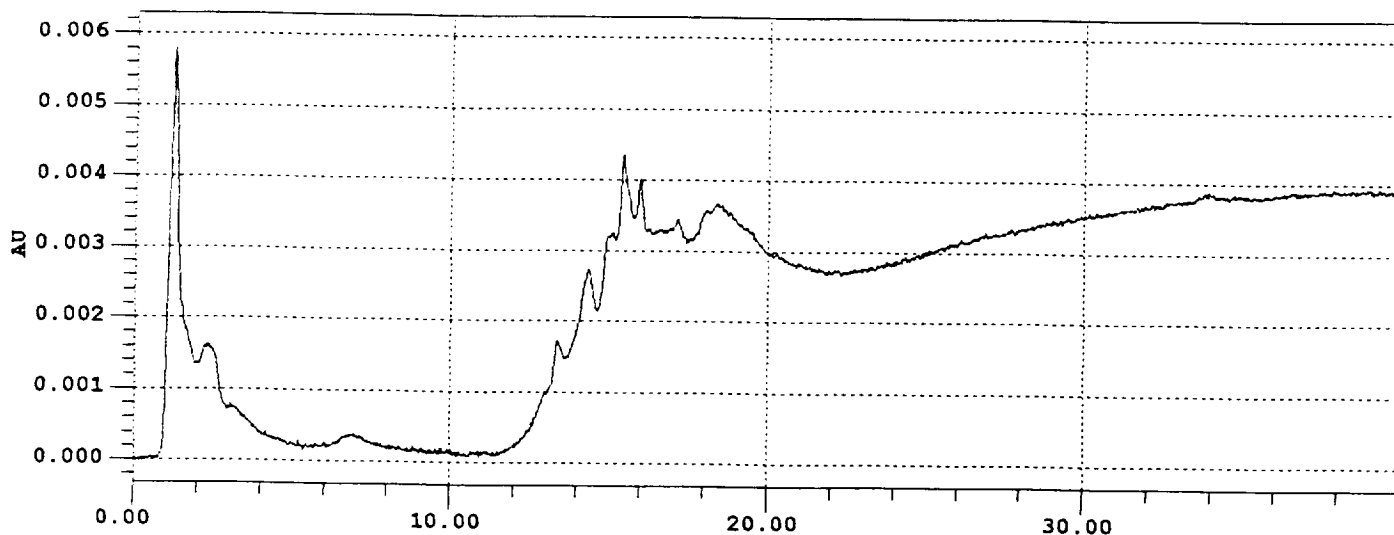
Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

For Sample: cck3fr31-33Fio Vial: 3 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 10:11 AM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | cck3fr31-33Fio | Volume: | 200.00 |
| Vial: | 3 | Run Time: | 45.0 min |
| Injection: | 1 | Date Processed: | 03/02/95 10:11 AM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 03/02/95 09:24 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |



Peak Results

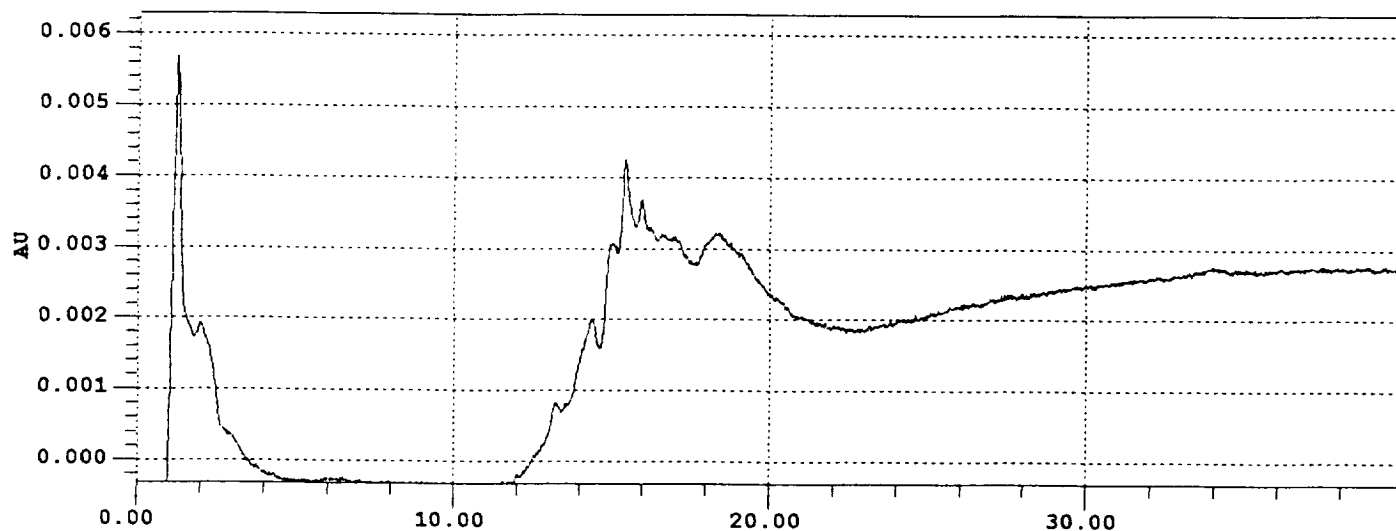
| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

For Sample: cck3fr36-37Fio Vial: 4 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 11:02 AM

Millennium Sample Information

Project Name: kris
 Sample Name: cck3fr36-37Fio
 Vial: 4
 Injection: 1
 Channel: 490 Ch1
 Date Acquired: 03/02/95 10:16 AM
 Scale Factor: 1.00
 Acq Meth Set: kris_ms
 Processing Method: kris_pm

Sample Type: Unknown
 Volume: 200.00
 Run Time: 45.0 min
 Date Processed: 03/02/95 11:02 AM
 Dilution: 1.00000



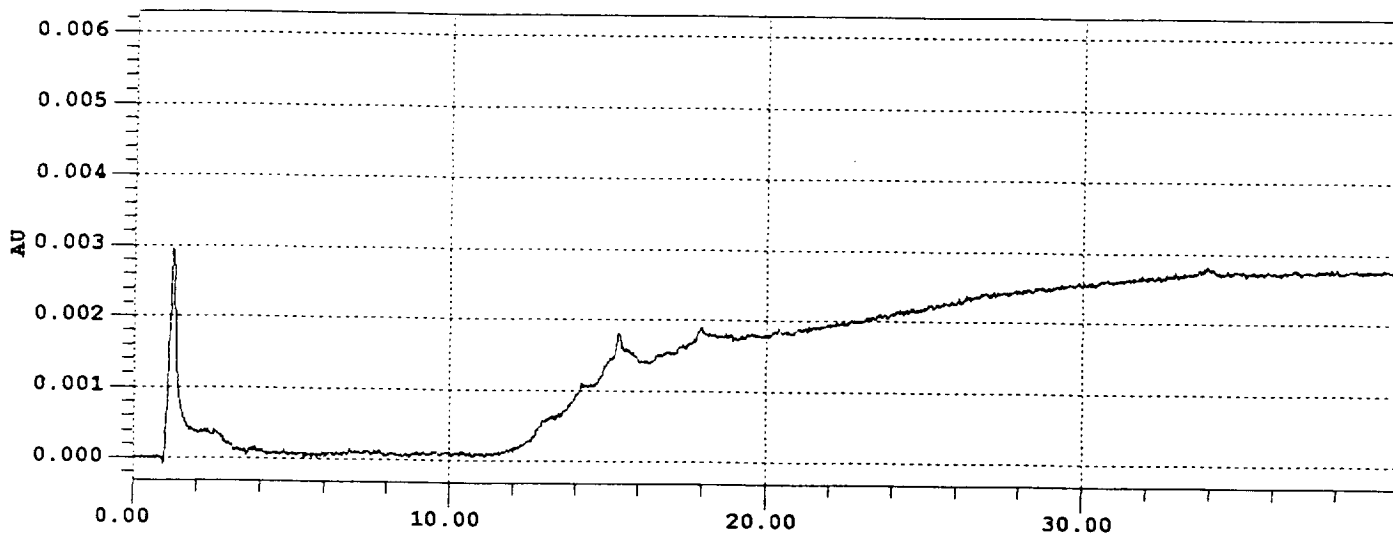
Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

For Sample: cck3fr34-35G10 Vial: 5 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 12:00 PM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | cck3fr34-35G10 | Volume: | 200.00 |
| Vial: | 5 | Run Time: | 45.0 min |
| Injection: | 1 | Date Processed: | 03/02/95 12:00 PM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 03/02/95 11:14 AM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |



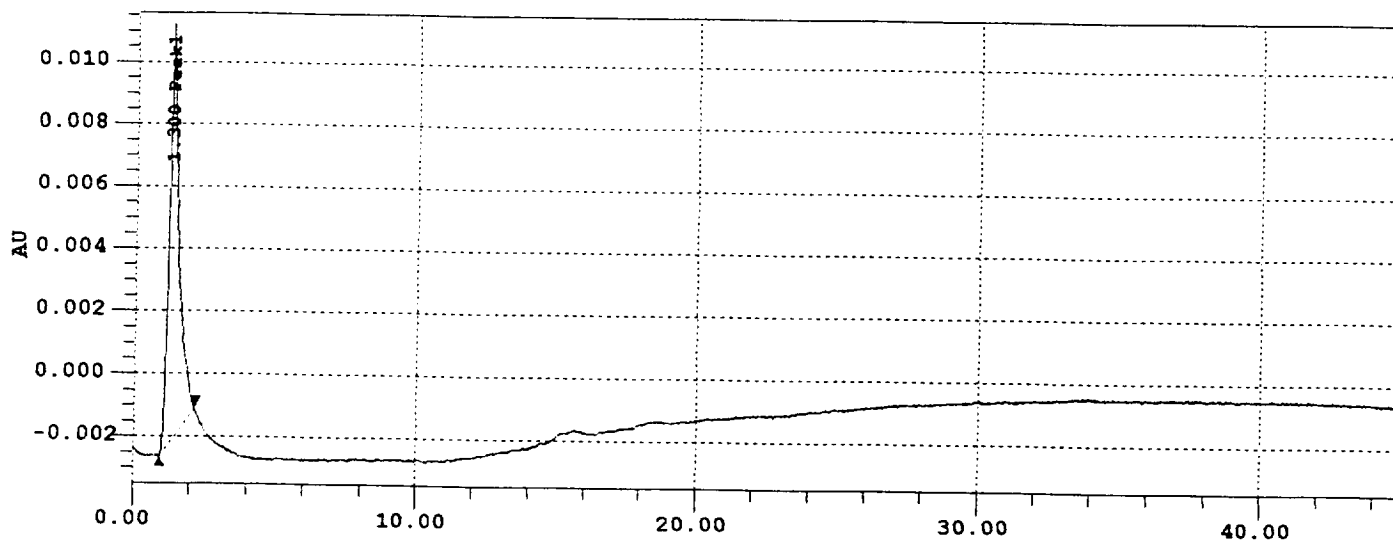
Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

For Sample: cck3fr36-37G10 Vial: 6 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 02:19 PM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | cck3fr36-37G10 | Volume: | 200.00 |
| Vial: | 6 | Run Time: | 45.0 min |
| Injection: | 1 | Date Processed: | 03/02/95 02:19 PM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 03/02/95 12:02 PM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | 36g | | |



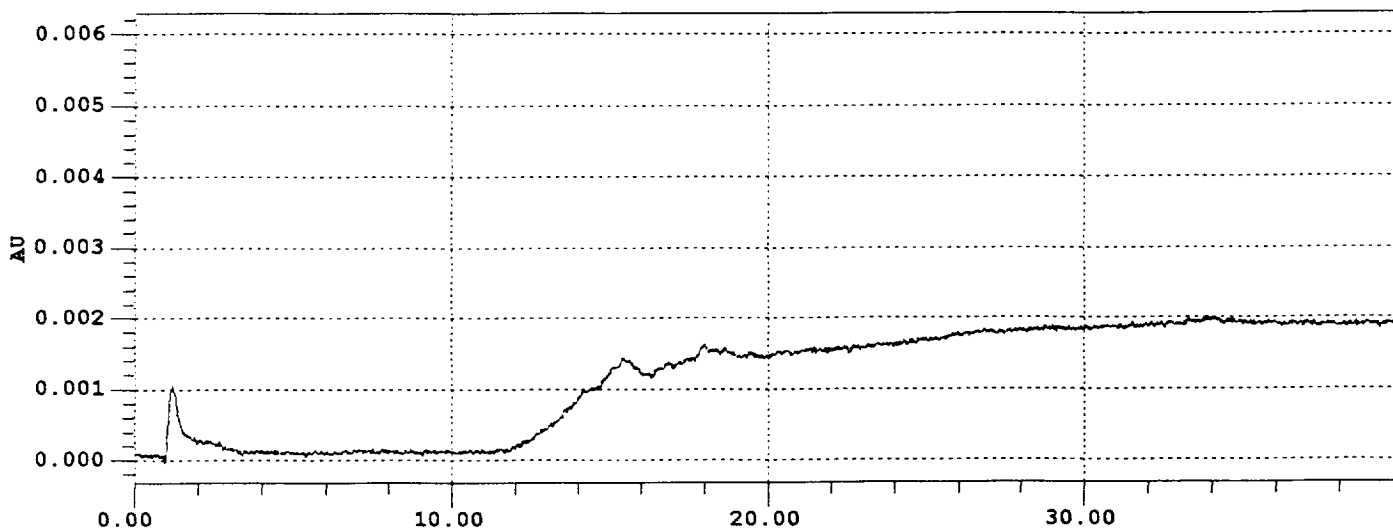
Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|----------------|---------------|-------------|--------|----------|
| 1 | Peak1 | 1.300 | 312085 | 13110 | | BB |

For Sample: cck3fr38Gion Vial: 1 Inj: 1 Chan: 490 Ch1 Date Processed 03/02/95 01:47 PM

Millennium Sample Information

| | | | |
|--------------------|-------------------|-----------------|-------------------|
| Project Name: | kris | Sample Type: | Unknown |
| Sample Name: | cck3fr38Gion | Volume: | 200.00 |
| Vial: | 1 | Run Time: | 45.0 min |
| Injection: | 1 | Date Processed: | 03/02/95 01:47 PM |
| Channel: | 490 Ch1 | Dilution: | 1.00000 |
| Date Acquired: | 03/02/95 01:01 PM | | |
| Scale Factor: | 1.00 | | |
| Acq Meth Set: | kris_ms | | |
| Processing Method: | kris_pm | | |



Peak Results

| # | Name | Ret Time (min) | Area (uV*sec) | Height (uV) | Amount | Int Type |
|---|-------|-------------------|------------------|----------------|--------|----------|
| 1 | Peak1 | 1.033 | | | | Missing |
| 2 | Peak2 | 2.717 | | | | Missing |
| 3 | Peak3 | 4.817 | | | | Missing |

Summary of CCR #3. Results to Date: The Bottom Line and Tentative Conclusions.

Several lines of evidence offer support for the idea that the FFEU was separating pituitary GH as well as general pituitary protein that was contained in a fresh cell lysate that was prepared by the astronaut. These include the observations that:

- bandsread and number of GH peaks was greater in the flight sample (pg. 8).
- Western blot analysis show some differences in iGH forms contained in different flight fractions after SDS-PAGE under non-reducing conditions (pg. 9).
- protein profiles of material in the 4 FFE flight fractions, after HPLC-gel exclusion chromatography, show some differences.

It is also important to indicate that flight had a definite effect on intracellular protein profile in the original lysate (before electrophoresis) - pg. 10C. These differences tended to be in areas of higher molecular weight protein. This result implies that microgravity affected the amount of certain proteins inside the cell. This change became apparent, in part, because of in-flight processing steps done with cells in CCK #3.

V. CCK #4

CCK #4

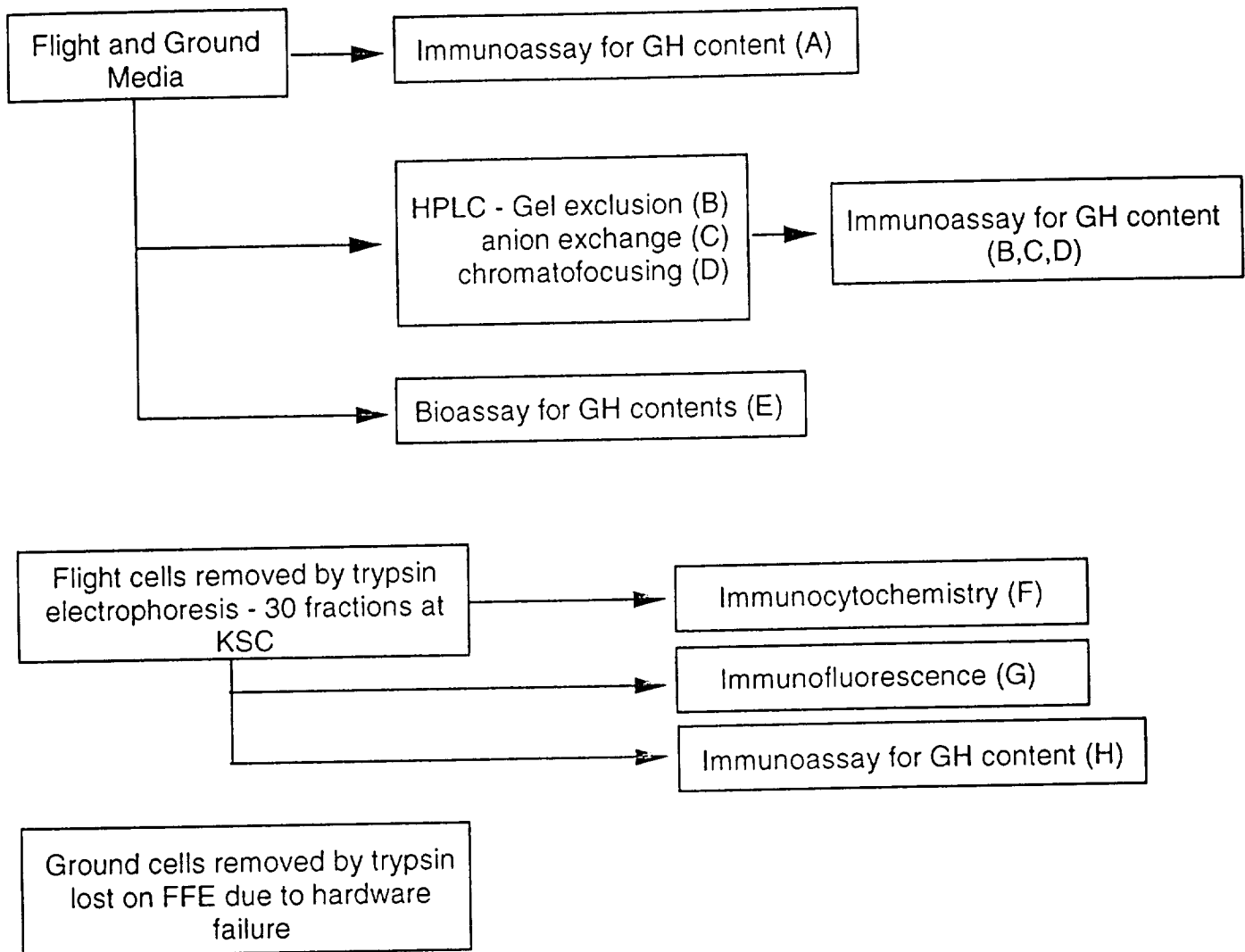
The original purpose for the cells in this CCK changed due to FFE hardware problems; however it did serve an extremely useful purpose for other parts of this experiment. For example, it provided comparative data for the cell culture portion of this experiment (detailed in section describing CCK #1 data).

Other CCK #4 results show:

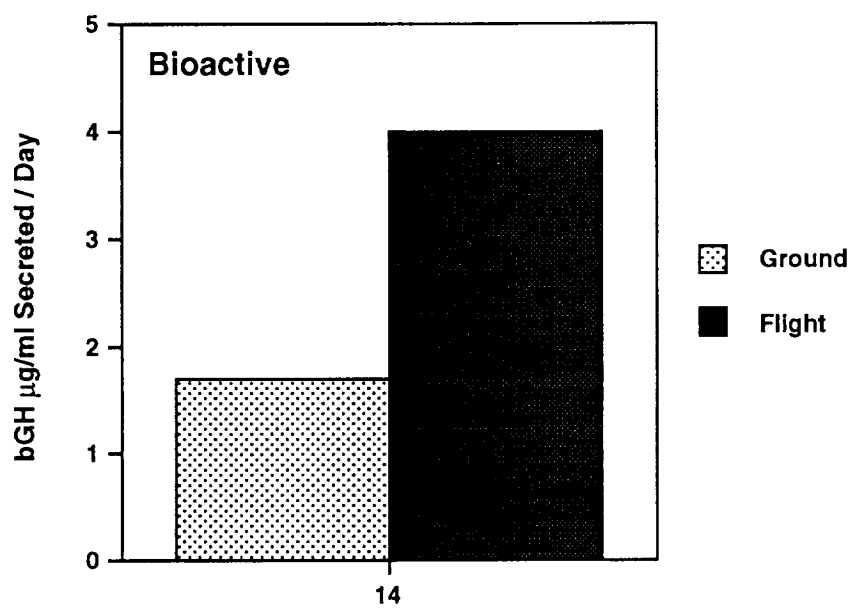
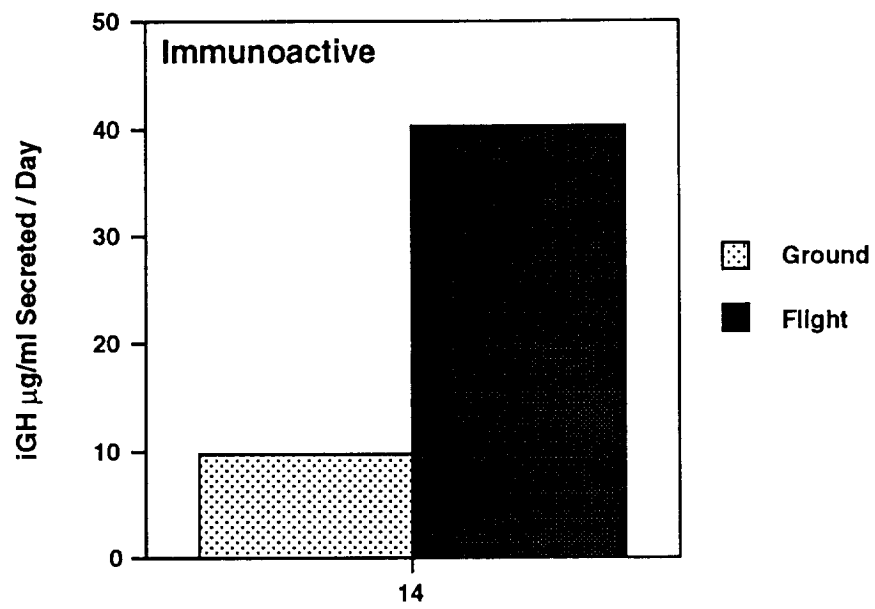
- That not only was there more iGH in media from this CCK than from the other CCK's, there was also 3x more iGH in the flight sample (pg. 4).
- That the electrophoretic mobility of the cells in CCK #4 is much lower than those from CCK #1 (pg. 5) with little evidence for separation of GH cells. Low mobility is tentatively attributed to lack of media change.
- That high intracellular GH in Fr. #30 (see pg. 5 bottom) correlates with the distribution profile showing a peak of GH cells in this fraction (pg. 5 top)
- That fibroblast growth in CCK #4 is minimal (both ground and flight) as determined microscopically and by flow cytometry. Photomicrographs of cells in CCK #1 and #4 immediately on return from space were included in an early report (given in Germany). The flow cytometry profiles of cells from CCK #4 (pgs. 6 and 7) again reflect the major effect of media change on relative lack of fibroblast growth and large numbers of cells with >2C contents of DNA compared to their CCK #1 counterparts (pg. 11/12, CCK #1).
- That the significant effect of spaceflight on cytoplasmic area occupied by GH is most easily seen in CCK #4 (pg. 8). We believe that the decrease in cytoplasmic area occupancy could be explained by the polycarbonate surface of the CCK. Note that this decrease does not occur on conventional tissue culture plasticware surface. How these biophysical changes relate to GH production and secretion remains a question.

| Proposed | Actual | % of objective achieved |
|---|---|-------------------------|
| CCK #4 | | |
| <ul style="list-style-type: none"> Cells maintained in culture for duration of flight without media changes. | Cells maintained in culture for duration of flight without media changes. | 100% |
| <ul style="list-style-type: none"> FFE of cells at KSC on return to earth. | FFE of cells at KSC on return to earth. | 100% |
| <ul style="list-style-type: none"> Synchronous ground control for FFE separation. | Hardware failure at KSC prohibited cell separation of ground sample by FFE. | 10% |

CCK #4 Growth Hormone Analysis



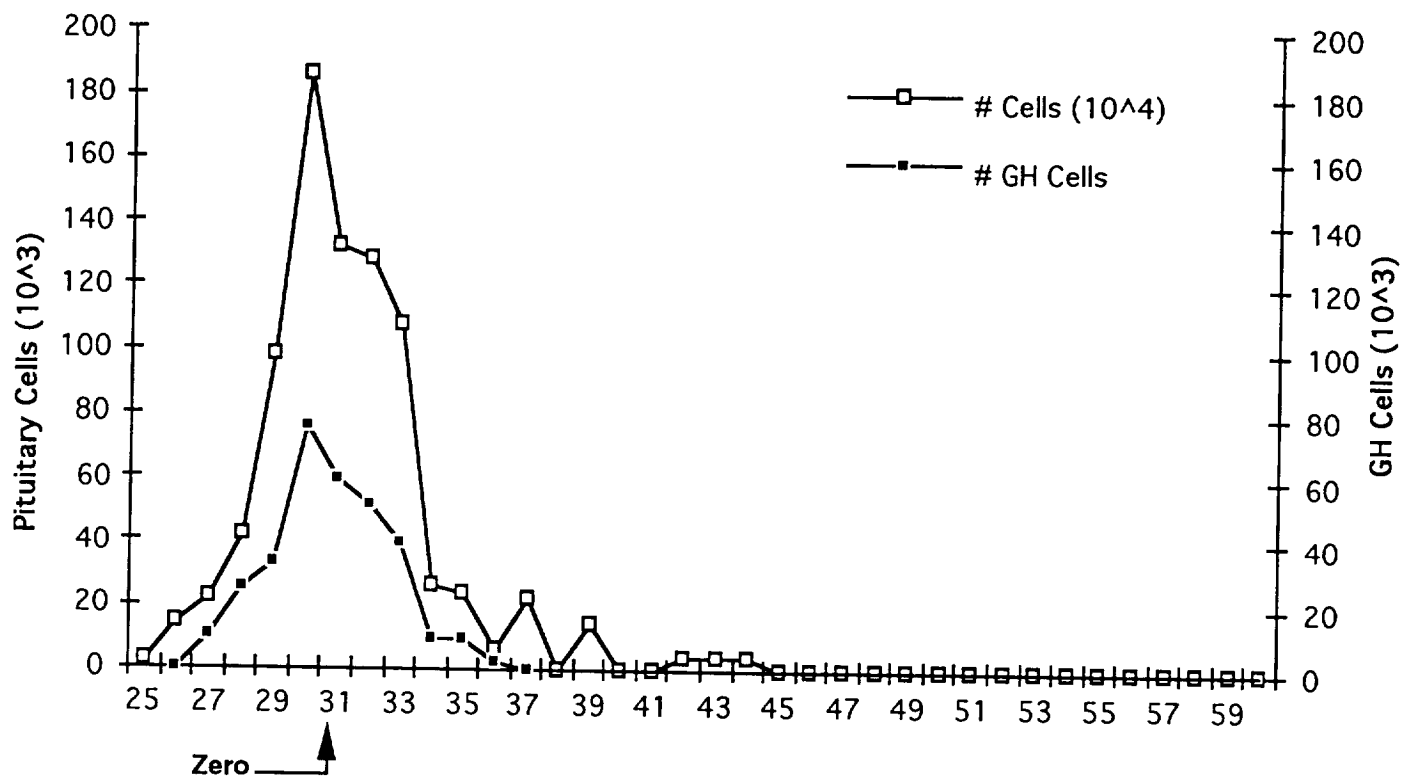
CCK 4 Media Recovered



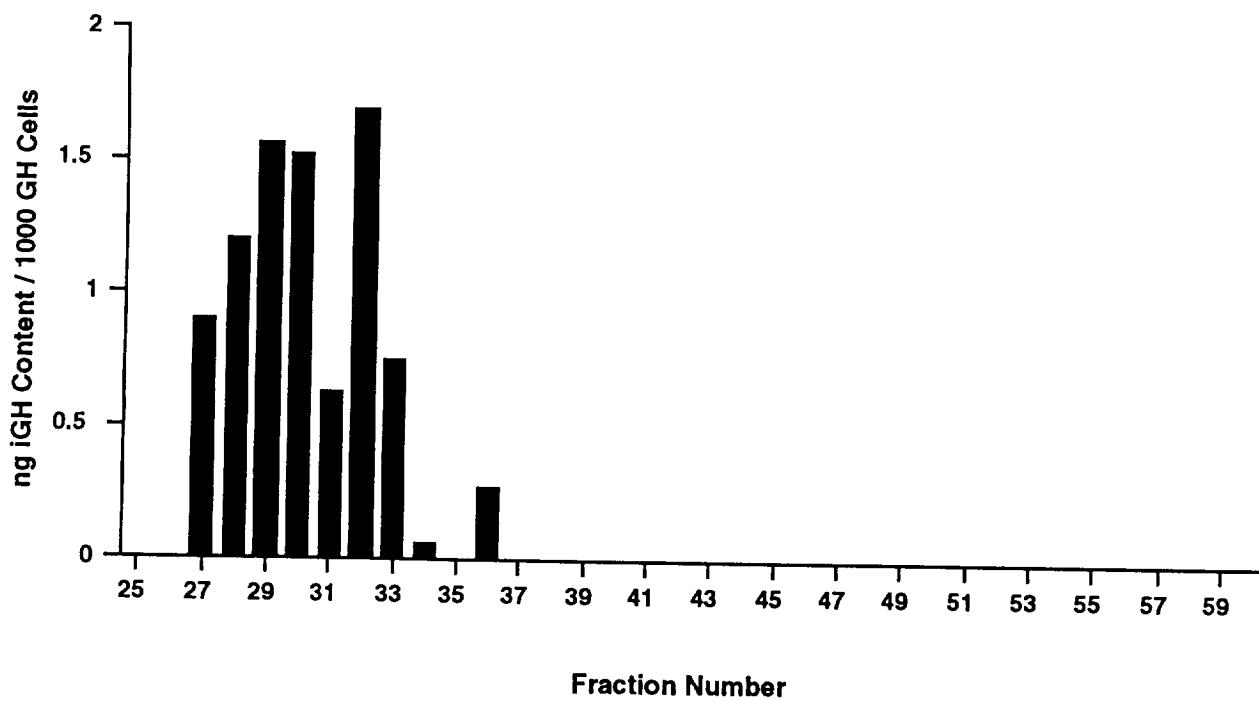
Mission Day

***Note Scale Difference**

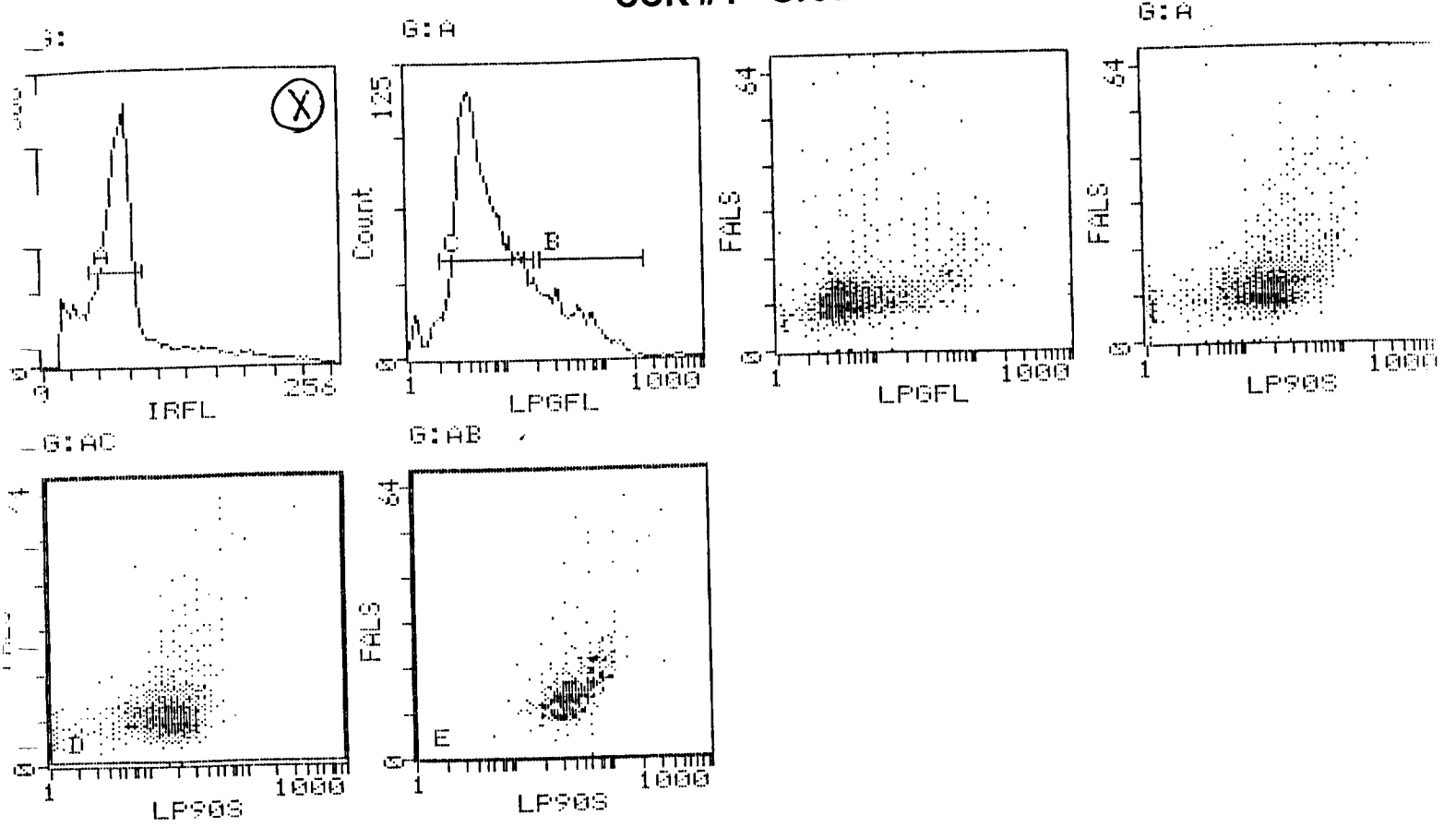
Flight CCK 4 Pituitary Cell Run Profile from ground Free Flow Electrophoresis Unit



Intracellular Growth Hormone Content of CCK 4 Electrophoresed Pituitary Cell Fractions



CCK #4 - Ground



----- SAMPLE INFO -----

| | | | | | |
|---------------|----------|-------------|---------|-------------|---------------|
| ROTOCOL | :TOM | TOTAL COUNT | :10,001 | LAST HIST | : |
| ATA RATE | :Unknown | | | LIST FILE | :251094P4.L05 |
| INSTRUMENT | :MDADS | | | PROT FILE | :T0000007.PRO |
| SAMPLE NAME | :IML2 | | | SAMPLE DATE | :250ct94 |
| SAMPLE NUMBER | :5 | | | SAMPLE TIME | :00:27 |
| OMMENTS | :G4P | | | | |

```

----- STATISTICS -----
                        DUAL PARAMETER STATISTICS
-----
ID  Pcnt  Area  .....Peak.....  ....X Channel....  ....Y Channel....
      Mean      SD      CV      Mean      SD      CV
1   100   4999   19, 10      67   14.2   11.2   78.8   12.2   7.5   61.3
2   100   1254   40, 13      13   41.3   27.8   67.2   16.7   9.5   56.6

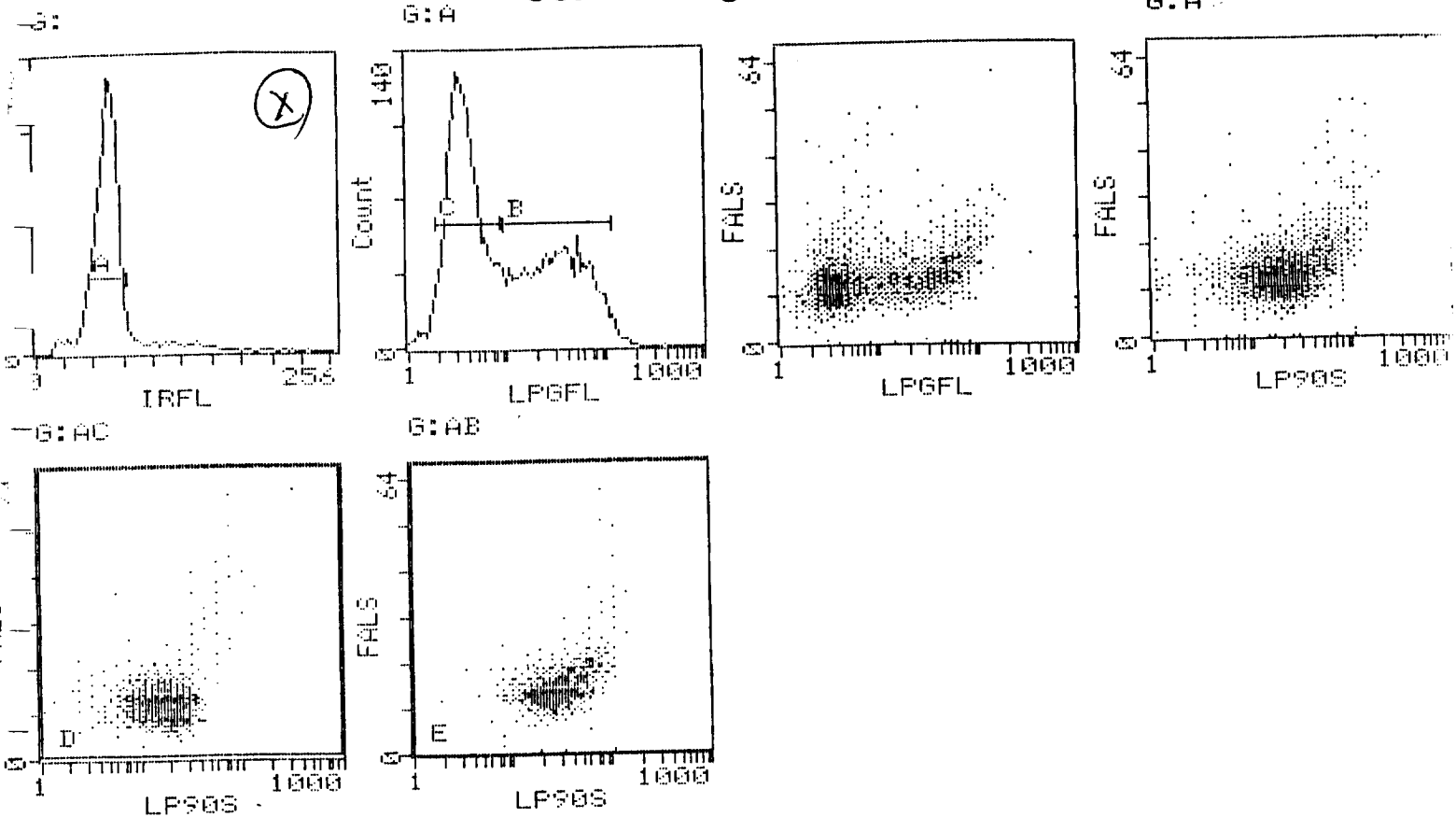
D  Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D
E  Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

```

FILE NAME: IML2

CCK #4 - Flight

A (38)



SAMPLE INFO

PROTOCOL : TOM
 DATA RATE : Unknown
 INSTRUMENT : MDADS
 SAMPLE NAME : IML2
 SAMPLE NUMBER : 7
 COMMENTS : F4P

TOTAL COUNT : 10,001

LAST HIST :
 LIST FILE : 251094P4.L07
 PROT FILE : T0000007.PRO
 SAMPLE DATE: 250ct94
 SAMPLE TIME: 00:33

STATISTICS

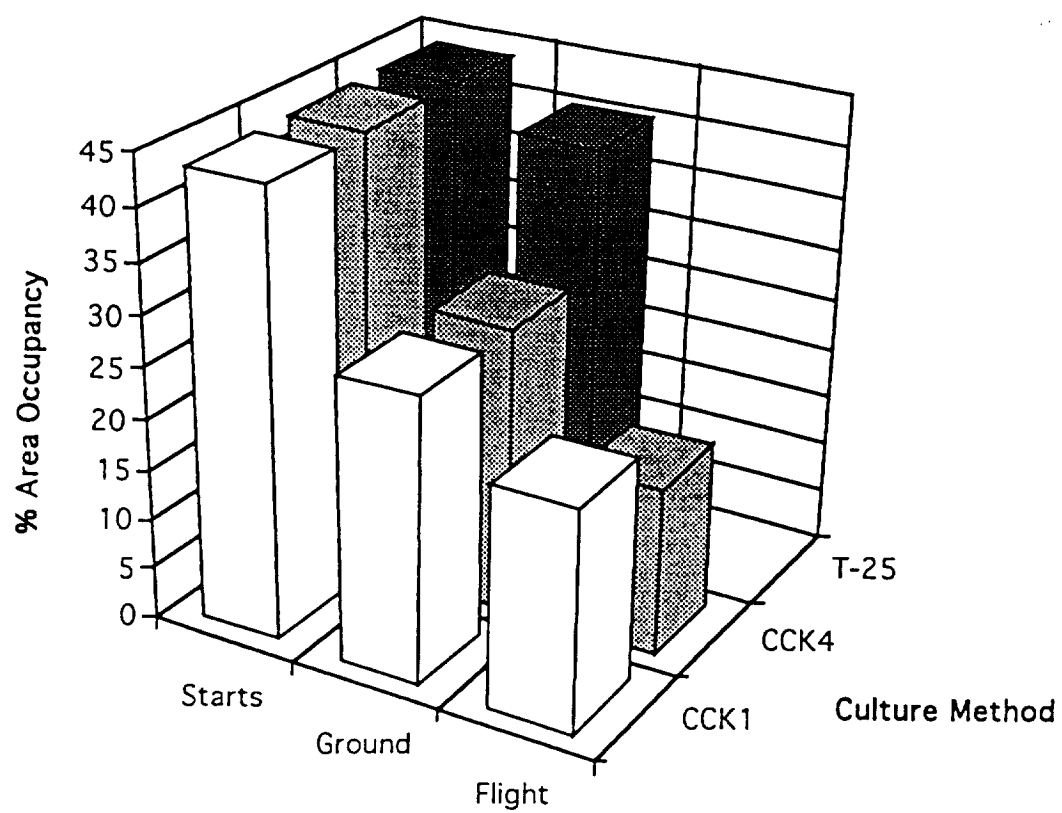
DUAL PARAMETER STATISTICS

| ID | Pcnt | Area |Peak..... | |X Channel.... | | |Y Channel.... | | |
|----|------|------|----------------|--------|-------------------|------|------|-------------------|-----|------|
| | | | Position | Height | Mean | SD | CV | Mean | SD | CV |
| 1 | 100 | 4235 | 21, 12 | 49 | 15.6 | 11.1 | 71.3 | 12.9 | 6.0 | 46.4 |
| 2 | 100 | 3384 | 19, 12 | 28 | 29.4 | 20.0 | 67.9 | 14.8 | 5.7 | 38.1 |

Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 D

Low X = 1.0, High X = 1024, Low Y = 0, High Y = 63 E

IML-2 Pituitary Cell Area Occupancy



Summary of CCK #4. Results to Date: The Bottom Line and Tentative Conclusions.

Lack of media change had marked consequences on pituitary cell structure and function. Some of these differences were apparent between ground and flight whereas these were not.

- The major difference between cells in CCK #4 vs. #1 was that fibroblast growth was minor in CCK #4 (this was true for both ground and flight).
- Electrophoretic mobility of CCK #4 flight cells was very much less relative to fed cells.
- Flight cells in CCK #4 released 5x more immunoreactive GH than corresponding ground control cells in CCK #4 (pg. 16 of CCK #1 section).
- In order to try to keep cells releasing GH with good bioactivity in flight it appears that frequent media changes will be required.

VI. STATEMENT OF WORK

Statement of Work

NASA grant NAG 8-953

“Electrophoretic Separation of Cells and Particles from Rat Pituitary and Rat Spleen”

W.C. Hymer Principal Investigator
Professor of Biochemistry
006 Althouse Laboratory
Penn State University
University Park, Pa 16802
814 - 865 - 2407
814 - 863 - 3198

Period of Performance: 2/8/95 - 2/7/96

Electrophoretic Separation of Cell and Particles from Rat Pituitary and Rat Spleen (NAG8-953)

Statement of Work: 8 Feb. 1995 - 7 Feb. 1996

The objective for the next year's work is to complete the sample analysis outlined in the right hand box of the flow chart (fig. 1). We also need to perform the entire ground based experiment over again because of FFEU hardware failure. Thus we will do an asynchronous ground control experiment, utilizing the same hardware used in the flight experiment at KSC.

Tasks accomplished thus far are detailed in the mid-term progress report (submitted 3/17/95)

Tasks remaining to be accomplished:

CCK#1. Complete analysis of culture media changeouts for Growth Hormone (GH) bioactivity and Prolactin (Prl) immunoactivity and bioactivity. Analyze culture media from electrophoretically fractionated cells for GH and Prl immunoactivity. Perform image analysis on electrophoretically fractionated cells. Analyze extracts from cells for Heat Shock Protein (HSP) presence.

CCK #2. Complete analysis of culture media changeouts and cell lysate for GH bioactivity and Prl immunoactivity and bioactivity. Complete HPLC analysis of culture media and lysate for changes in GH. Perform FFE fractionation of cell lysate. Analyze cell lysate for HSP presence. Complete gel electrophoresis and Western blots of cell lysates for GH and Prl variant analysis.

CCK #3. Complete analysis of culture media and cell lysate for GH and Prl immunoactivity and bioactivity. Continue HPLC analysis of culture media for GH activity. Complete analysis of electrophoretically fractionated lysates. Perform gel electrophoresis and Western blot of cell lysate for GH and Prl variant analysis.

CCK #4. Complete analysis of culture media for GH bioactivity and Prl immunoactivity and bioactivity. Complete HPLC analysis of culture media for GH activity. Analyze cellular extracts for GH and Prl variants and HSP presence.

Asynchronous Ground Control . The requirement for an asynchronous ground control experiment is dictated by the failure of the Free Flow Electrophoresis unit postflight to process the ground control cells from CCK's 1 and 4 (detailed in mid-term progress report).

Prepare manuscripts and present data.